Reproductive isolation in smooth and palmate newts (Triturus vulgaris vulgaris and T. helveticus helveticus)

Thesis

REPRODUCTIVE ISOLATION IN SMOOTH AND PALMATE NEWTS (*TRITURUS VULGARIS VULGARIS* AND *T. HELVETICUS HELVETICUS*)

Julie Marie Roberts B.Sc.(Hons)

Submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy (Biology)

Brain and Behaviour Research Group
Department of Biology
The Open University

February 1990

Date of Submission : 13th February 1990
Date of award : 30th July 1990

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ABSTRACT

This thesis reports a study of reproductive isolation between two closely related sympatric British species of amphibian, the Smooth newt, *Triturus vulgaris vulgaris* and the Palmate newt, *T. helveticus helveticus*. Investigation shows that reproductive isolation is not attributable to differences in habitat, the timing of courtship or the failure of spermatophores to adhere to the cloaca of a heterospecific female and it is demonstrated that a proportion of F1 hybrid larvae are capable of survival beyond metamorphosis; by elimination, the lack of naturally occuring hybrids is primarily a consequence of sexual isolation.

Techniques for distinguishing both purebred and hybrid larvae and adult newts of both sexes and species based on differences in their external morphology are evaluated. The most suitable distinguishing characters (on which visual discrimination in newts may be based) are described. The variation in the courtship behaviour of *T. h. helveticus* is described in detail and compared to that of *T. v. vulgaris*. The similarity in form and transition of male acts suggests that qualitative differences in display and order of acts are not the primary source of information on which female discriminaton is based. Quantitative differences in the proportion of acts performed probably serve to emphasise species-specific cues which play a direct role in sexual isolation. A causal analysis is carried out on a relatively recently recognised *T. h. helveticus* display act, the wiggle. Evidence is presented that the function of this act is to re-attract a female that is becoming unreceptive and it does not appear to contribute to reproductive isolation.

Interspecific mating trials show that female *T. h. helveticus* are less receptive to heterospecific courtship than conspecific courtship. Male *T. v. vulgaris* display less vigorously to heterospecific females manipulated by means of a harness to perform positive behaviour than to conspecific females. It is demonstrated that male *T. h. helveticus* show selectivity towards conspecific females over heterospecific females on the basis of both visual and olfactory cues alone, whereas in male *T. v. vulgaris* and female *T. v. vulgaris*, the same preference is only observed on the basis of olfaction. When presented with an artifical fanning tail mimic, females of both species preferred the faster (*T. h. helveticus*) fan speed.

It is suggested that sexual isolation is the consequence of a dual system in which males fail to recognise, or are less receptive to the olfactory and/or visual cues produced by heterospecific females and consequently do not initiate courtship with them. Where interspecific courtship does occur, the stimulation produced by the male is inadequate to raise the receptivity of the female at a high enough rate for the encounter to progress to spermatophore transfer.

ACKNOWLEDGMENTS

This thesis would not have been possible without the assistance of many people. In particular I would like to thank Steve Arnold, Tim Halliday, Richard Griffiths and Paul Verrell for their helpful discussion and Miriam Griffiths and Verina Waights for technical assistance. I would also like to thank my 'minders', Angela Buckley, Martin Cambrey-Deakin and Charlie Studdy; and all those in the Academic Computer Support Department of the Open University who guided me in computing and statistics. I would also like to mention John Baker, Andrew Bielinski, Kevin Chapman and Lancaster University, Arnold Cooke and the Nature Conservancy Council staff at Castor Hanglands National Nature Reserve, Christina Giacoma, Lottie Hosie, Lynne Houck, Gary Kennedy, Tony King, Norah McCabe, Herbert MacGregor, Marion Petrie, Caroline Pond, Chris Raxworthy, my parents - Lillian and Maurice Roberts, Simon Sims, Carl Taylor, Pieter De Wijer and many others in the Biology Department of the Open University who have provided a wealth of advice, support and assistance. Special thanks are extended to Tim Halliday, who conceived this project and whose continual encouragement has been so important.

Financial support for this studentship was provided by the Science and Engineering Research Council.

DEDICATION

This thesis is dedicated to my grandmother Emily, who hoped that one of the family would go to university; to my daughter Emma, who has put the world in perspective; and to Wally, the best thing in Milton Keynes.

LIST OF CONTENTS

LIST OF FIGURES

Figure **Legend**

LIST OF PLATES

Plate **Legend**

LIST OF TABLES

4.6 The number of courtship display acts performed during the most vigorous trials with conspecific and heterospecific females.

4.7 Partner responsible for ending the last encounter of a trial.

4.8 Duration spent by male *T. v. vulgaris* in proximity to 'receptive' and 'unreceptive' conspecific and heterospecific females.

4.9 Number of positive acts performed by male *T. v. vulgaris* towards 'receptive' and 'unreceptive' conspecific and heterospecific females.

4.10 Median number of sniffing bouts performed by male *T. v. vulgaris* towards 'receptive' and 'unreceptive' conspecific and heterospecific females.

5.1 The trials carried out.

5.2 Duration spent near conspecific and heterospecific visual stimuli.

5.3 Duration spent near heterospecific and blank stimuli.

5.4 Duration of visits to conspecific and heterospecific females by sexually motivated males.

5.5 A comparison of the behaviour of sexually motivated males within 4.5cm of conspecific and heterospecific females.

5.6 Discrimination based on olfactory cues alone.

5.7 Discrimination in newts showing evidence of olfaction.

5.8 The response of female *T. v. vulgaris* and *T. h. helveticus* to a tail mimic set to conspecific and heterospecific male fan display modes.

5.9 Number of spermatophores deposited in response to positive behaviour by females which earlier approached a tail mimic compared to those that did not.

CHAPTER 1

REPRODUCTIVE ISOLATION MECHANISMS

The classification of organisms into species has provided a useful practical tool for biologists for decades. Although subject to criticism (e.g. Ehrlich 1961, Lambert *et al.* 1987, Sokal & Crovello 1970), it is the orthodox view (Coyne *et al.* 1988, White 1978) that sexually reproducing species can be defined in terms of the Biological Species Concept. According to Mayr (1940, 1963) species may be defined as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups". Futuyma (1979) wrote that "if the Biological Species Concept is accepted, species are best recognised by charactersistics which prevent their interbreeding .... called isolating mechanisms." The importance of distinguishing reproductive isolation, in which interbreeding of two populations is prevented by intrinsic mechanisms (which is fundamental to the biological species concept), from geographical isolation, which is dependant on extrinsic factors, has been stressed by Mayr (1976).

It was Dobzhansky (1937) who first used the term 'isolating mechanisms' and later defined them as any genetically conditioned impediment to gene exchange between populations (Dobzhansky *et al.* 1977). Dobzhansky (1937) classified them into two main groups: a) mechanisms which prevent production of hybrids, or prevent them reaching sexual maturity and b) the production of sterile hybrids. Mecham (1961) further advanced the classification of isolating mechanisms by dividing them into pre- and post-mating mechanisms. Mayr (1963) noted a fundamental difference between the two types and concluded that the former are highly susceptible to improvement by natural selection as they prevent wastage of gametes,

whilst the improvement of the latter by natural selection would be 'difficult and indirect' as they involve wastage of gametes. Barton and Hewitt (1985) take reproductive isolation to mean complete absence of gene flow between species, with no fertile hybrids being formed, but both Mayr and Dobzhansky recognised that absolute reproductive isolation cannot be used as a criterion for species distinction as occasional hybridisation occurs between species that normally maintain different gene pools (White 1978). The following is Mayr's (1963) classification of isolating mechanisms:-

---

1. ***Mechanisms that prevent interspecific crosses (premating mechanisms).***
   a)Potential mates do not meet (seasonal and habitat isolation).
   b)Potential mates meet but do not mate (ethological isolation).
   c)Copulation attempted but no transfer of sperm takes place (mechanical isolation).

2. ***Mechanisms that reduce the full success of interspecific crosses (postmating mechanisms).***
   a)Sperm transfer takes place but egg is not fertilized (gametic mortality).
   b)Egg is fertilized but zygote dies (zygote mortality).
   c)Zygote produces an F1 hybrid of reduced viability (hybrid inviability).
   d)F1 hybrid zygote is fully viable but partially or completely sterile, or produces deficient F2 (hybrid sterility).

Table 1.1 Classification of isolating mechanisms (After Mayr 1963).

Based on Muller's (1942) classification, Liley (1966) suggested that a more valid distinction would be pre-fertilization and post-fertilization mechanisms, whilst Littlejohn (1969) proposed a more complicated classification, with the addition of the categories hybrid ethological isolation and hybrid breakdown.

The origin and evolution of biological isolating mechanisms has been the subject of much discussion in the literature. Muller (1939) suggested that isolating mechanisms primarily arise as a by-product of the general evolution of populations. Mayr (1963) was one of many to discuss this further and he proposed that the primary cause of speciation was a product of general evolutionary divergence during spatial separation (allopatric speciation), which must be efficient when contact between two populations is first established, although he recognised that natural selection may result in the aquisition of secondary isolating mechanisms. Dobzhansky (1951) advocated that isolating mechanisms do not necessarily develop fully until the populations overlap geographically (sympatric speciation), when natural selection pressures develop and strengthen isolating mechanisms by selecting against discordant gene patterns in interbreeding populations and by favouring recognition of distinctive species traits. This process has been called reinforcement by Blair (1955).

Liley (1966) considered three predictions arising from the reinforcement hypothesis. In brief:

1) Isolating mechanisms which are selected for by reinforcement are likely to be more efficient than those that are simply by-products of divergence.

2) A number of factors may impede interbreeding, but only a limited number of them may be reinforced.

3) Selection will favour those potential isolating mechanisms which either operate at an early stage in pre-fertilization procedures, or reduce the possibility of populations meeting during the reproductive period.

He surmised that ethological isolating mechanisms were among the most likely to be selected for, because they would operate early in the mating process.

Tinbergen (1953) classified the functions of mating behaviour and among them listed reproductive isolation, ensuring that only individuals of the same species mate with one another. The others included synchronisation between partners in the timing of mating, orientation so that the male can find the female (or *vice versa*) and persuasion, in which the male stimulates the female to mate. A fifth function, assessment of a conspecific as a suitable mating partner, has been added by Trivers (1972). Differences in reproductive behaviour between species involve evolutionary divergence in such characters at some stage in the speciation process, either during allopatry, parapatry or sympatry. Paterson (1985) stressed that reproductive systems such as courtship signals function primarily to achieve efficient mating and fertilization within species and are therefore subject to stabilizing selection, where individuals with extreme characters are not favoured. He used the term 'specific mate recognition system' to describe the set of characters responsible for this co-adaptation.

Differences in the specific mate recognition systems which result in the failure of potential interspecific encounters (where the heterospecific is not recognised as an appropriate mating partner) are believed to arise during allopatric differentiation as a consequence of adaptation of these recognition systems to different local habitats (Paterson 1985, Verrell 1988). However, Verrell (1989) points out that pleiotropic effects of local adaptation in other characters (Muller 1942), random fixation of 'incompatibility alleles' (Nei *et al.* 1983), genetic transiliences (Carson & Templeton 1984) and sexual selection in isolated populations (Lande 1981) may also be important.

When applied to evolutionary pathways leading to divergence in mate recognition systems, Butlin (1987) advocated that the term 'reinforcement' should be restricted to those cases in which partially inviable or sterile hybrids are produced. Here, he argued, the selection process is genuinely a part of speciation, as it may act to reduce gene flow between populations in parapatry, sympatry or in a cline, by increasing assortative mating. He distinguished this process from selection to favour the reduction of wastage of reproductive effort in populations which produce completely inviable or sterile hybrids, as the lack of gene flow between populations indicates that the speciation process is complete. For the latter process he proposed the term 'reproductive character displacement', because he regarded it as an interspecific interaction comparable with ecological character displacement (Brown & Wilson 1956, Grant 1975). In his critique of speciation by reinforcement, he cautions that few examples have been detected and that reinforcement is difficult to demonstrate unequivocally. Other modes of speciation are discussed at length by White (1978).

THE AIMS OF THE STUDY

This thesis forms a quantitative laboratory study intended to extend Halliday's (1977a) preliminary investigations on reproductive isolation mechanisms in two British species of newts, the Smooth newt, *Triturus vulgaris vulgaris* (Linnaeus 1758) and the closely related Palmate newt, *T. helveticus helveticus* (Razoumowsky 1789). It represents a complementary study to that of Raxworthy (1989a) on European species and sub-species of *Triturus*.

A SHORT NATURAL HISTORY OF THE NEWT

Descriptions of the natural history of *T. v. vulgaris* and *T. h. helveticus* have been given by Bell (1973), Halliday (1972), Smith (1973), Steward (1969) and Verrell (1983), hence only a brief account with additional information is provided here. Newts begin their life in water and after many months as aquatic larvae they metamorphose and migrate onto land. The terrestrial larvae (efts) remain on land until they reach maturity and thereafter enter a cycle of migration to and from water in the spring and autumn, respectively. Adult *T. v. vulgaris* tend to be philopatric, returning to the same breeding site in successive seasons (Steward 1969, Dolmen 1981). At the beginning of the Spring migration, which usually begins in Britain around March (Harrison *et al.* 1983, Verrell & Halliday 1985) the newts become re-adapted for their temporary aquatic existance. These re-adaptations include modification of the sensory organs and skin (Halliday 1972), development of integumental characters (Griffiths & Mylotte 1989, Verrell *et al.* 1986) and changes in the internal reproductive organs (Verrell *et al.* 1986).

Griffiths (1985) found that diel mating activity in *T. v. vulgaris* was mainly crepuscular and peaked predominantly around dusk. Males of both species have an elaborate courtship display (Halliday 1977a) and transfer sperm externally to the female by means of a spermatophore; fertilization is internal. Eggs are wrapped by the females in the leaves of aquatic vegetation, but no further parental care is given by either partner. Verrell and McCabe (1988) reported that a female lays only a proportion of her egg compliment after insemination with one spermatophore although more than one may be transferred (Halliday 1974) and females may mate with more than one male (Verrell 1984). During any one breeding season females have a limited number of yolked oocytes to lay (Verrell *et al.*

1986) and as a consequence of the way sperm is produced (post-nuptial spermatogenesis), male newts also have a limited number of gametes available (Lofts 1984, Verrell *et al.* 1986). Once a male has deposited several spermatophores, a latency of 24 to 48 hours is usually required before he can lay down the same number in successive courtships (Halliday 1976, Verrell 1986a).

Part of the aquatic phase may not only be devoted to breeding, but it may also be a time to feed and increase body mass before the winter months of torpidity (Verrell & Halliday 1985). Toward the end of the aquatic phase re-adaptation for terrestrial life takes place and adult migration from the water in England may occur at any time between the months of June and December (Verrell & Halliday 1985).

REPRODUCTIVE ISOLATION MECHANISMS IN AMPHIBIANS

Anurans

Several authors have demonstrated that female anurans are attracted to calling conspecific males (Blair & Littlejohn 1960, Littlejohn 1965, Littlejohn & Loftus-Hills 1968), and Michaud (1962) and Snyder and Jameson (1965) have shown that female anurans exhibit preferential selectivity for the call of conspecific males over those of closely related sympatric species. In Hylid (Ball & Jameson 1966) and Chorus frogs (Fouquette 1975) difference in mating call is thought to be the principal factor responsible for pre-mating reproductive isolation. Passmore (1981) discussed the relevance of vocalisation to the specific mate recognition concept proposed by Paterson (1978) and advocated that ethological isolation is a consequence of the attractant role of the call in promoting conspecific mating (i.e. sexual selection) rather than selection to limit hybridisation being the primary factor leading to call divergence. Both body size and

temperature are factors which may influence the dominant frequency of anuran mating calls, but neither are currently thought to play a role in species discrimination by Hylid frogs (Ball & Jameson 1966).

Despite reports of distinct variation in morphology between some related sympatric species (e.g. *Scaphiopus*), the role of visual cues in anuran species discrimination has been poorly studied and the possible role of olfactory cues has not been subject to empirical investigation (Madison 1977). Kondrashev *et al.* (1976) showed that male *Rana temporaria* prefered to clasp red models and compared this to two species of *Bufo* which preferred black models. They concluded that the existence of chromatic sexual dimorphism in *Rana temporaria* during the breeding season accounted for the preference shown by males of this species, but their data suggest that this visual preference may contribute to pre-mating isolation between *Rana* and *Bufo* species. Pre-mating isolation may be maintained by a combination of mechanisms which may or may not include auditory cues (Blair 1964). In frogs of the *Rana pipiens* complex, temporal spacing of the breeding cycle is also important (Frost & Bagnara 1977, Frost & Platz 1983, Hillis 1981) and in Texan toads of the genus *Bufo* and Spadefoot Toads (*Scaphiopus*) both differential habitat selection and partial segregation of the breeding phase appear to be prominent pre-mating isolation factors.

Post-mating mechanisms may also limit the occurrence of hybrids in zones of sympatry. Developmental abnormalities occur in a large proportion of hybrid progeny derived from a female *Litoria ewingi* x male *L. paraewingi* cross but progeny of the reciprocal cross develop normally to metamorphosis (Watson 1972). A similar asymmetry in larval viability has been demonstrated in reciprocal crosses between *Xenopus wittei* and *X.*

laevis (Tinsley *et al.* 1979). Tinsley *et al.* (1979) however, found that both the crosses of *X. wittei x X. vestitus* produced viable offspring, but mature male hybrids were found to be sterile and although female F1 hybrids backcrossed with parental stock and produced fertilized eggs, the offspring usually died during embryogenesis.

Urodeles

Reproductive isolation is potentially an important factor in the evolution of courtship differences between species because of the possible role of species-specific displays as ethological isolating mechanisms (Halliday, in press). However, it is a topic that has been relatively poorly studied in urodeles (Houck *et al.* 1988).

Kawamura and Sawada (1959) and Sawada (1963a) investigated ethological isolation in morphologically distinct (Sawada 1963b) local races of Japanese newts of the genus *Cynops* and concluded that female discrimination of differences in courtship display was largely responsible for sexual isolation, although failure of synchronisation of the partners was also a contributing cause. Post-mating isolation mechanisms do not appear to operate as Kawamura (1950) reported that no significant inviability or sterility exists in F1 hybrids resulting from *Cynops pyrrhogaster x C. ensicauda* crosses.

Davis and Twitty (1964) thought that the minor differences in courtship display between *Taricha granulosa* and three sympatric congeners were insufficient to serve as reproductive isolating mechanisms; the results of earlier tests in which sponges soaked in female odours were used suggest that male discrimination of odours was a more important factor (Twitty 1961a). The success of artificial cross-fertilization (Twitty 1936, 1955,

Twitty & Bodenstein 1939) and the fertility of resulting hybrids (Twitty 1961b, 1964) further suggests that the very limited occurrence of *T. rivularis x T. granulosa* hybrids in the field is not a consequence of effective postmating mechanisms.

Complete, partial and no reproductive isolation has been established between a number of geographically isolated populations of *Plethodon jordani* and *P. glutinosus* with which they are sympatric (Highton & Henry 1970). Between non-hybridising populations, Dawley (1986, 1987) showed that male *P. glutinosus, P. jordani, P. aureolus, P. tayahalee* and *P. kentucki* all prefer to court conspecific females over heterospecific females, but showed that only male *P. tayahalee* from a hybridising location appeared to discriminate between conspecific and heterospecific females on the basis of odour. Male *Ambystoma jeffersonianum* show a similar conspecific preference when also presented with the odour of females of a unisexual, triploid congener *A. platineum* (Dawley & Dawley 1986). Dawley (1984, 1986) reports that the courtship behaviour of *Plethodon* species is very similar, but observed a clear preference for the odours of conspecifics shown by females when tested during reproductively active phases. This indicates that olfaction is probably an important pre-mating isolation mechanism in this genus.

Recognition of odour is also important in *Desmognathus*. Verrell (1989) found that male *D. imitator* and *D. ochrophaeus* prefered conspecific over heterospecific female odour and failed to court heterospecific females in 80% of courtship trials. He concluded that the production of inappropriate chemical cues by heterospcific females leads to a lack of response in males and that this process is responsible for the maintenance of sexual isolation in sympatric populations. Sexual isolation in *Desmognathus* has

also been studied by Houck *et al.* (1988) who found through mating trials, that nearly complete isolation exists between *D. ochrophaeus* and *D. fuscus* and that partial sexual isolation occurs between two populations of *D. ochropaeus* from Virginia and Tennessee. Verrell and Arnold (1989) suggest that discrimination between individuals from different populations is based on olfaction and that divergence of pheromones takes place in allopatry.

Reproductive isolation between *T. v. vulgaris* and *T. h. helveticus* is reviewed in the following section and so I will confine my discussion here to other *Triturus* species. Schoorl and Zuiderwijk (1981) suggest that differing habitat selection is largely responsible for reproductive isolation between populations of *T. cristatus* and *T. marmoratus*, which occupy a narrow zone of sympatry in France. However, where these species are syntopic, natural hybridisation (Arntzen 1986a, 1986b) may be partially attributable to sexual interference by female mimicry (Arnold 1972, Verrell 1984) by male *T. marmoratus* in *T. cristatus* courtships (Zuiderwijk & Sparreboom 1986). A hybrid zone is also reported to exist between *T. v. vulgaris* and *T. montandoni*, where hybrids are reported to make up 60% of the individuals in syntopic populations (Pecio & Rafinski 1985). The widespread differences in morphology and behaviour of these two species clearly do not function as an effective pre-mating barrier (Pecio & Rafinski 1985). The four species of the *T. cristatus* superspecies (*T. cristatus*, *T. dobrogicus*, *T. karelini* and *T. carnifex*) can be found in narrow contact zones (Raxworthy 1989a). However, natural hybridisation and gene exchange within this superspecies is limited (Wallis & Arntzen 1989). Laboratory crosses between these species show marked sterility barriers (Callan & Spurway 1951).

Accounts of natural hybrids are generally rare, but Schmidtler and Schmidtler (1967) report a possible *T. vittatus x T. karelini* hybrid. The most viable laboratory induced hybrids between *T. h. helveticus* and the subspecies of *T. vulgaris* are with *T. v. meridionalis* (Benazzi 1957, Freytag 1950); the numerous reports of artificially induced hybridisation between the various *Triturus* species and sub-species have been reviewed by Halliday (1977a). Several studies show that post-zygotic mechanisms operate, resulting in unfit or inferior hybrids (e.g. Callan & Spurway 1951, Lantz & Callan 1954, Scali & Mancino 1968).

REPRODUCTIVE ISOLATION MECHANISMS IN SMOOTH AND PALMATE NEWTS (*TRITURUS VULGARIS VULGARIS* AND *TRITURUS HELVETICUS HELVETICUS*).

Climatic changes during the Pleistocene are thought to have led to the divergence of the seven sub-species of *T. vulgaris* currently recognised (*T. v. vulgaris, T. v. schmidtlerorum, T. v. lantzi, T. v. meridionalis, T. v. ampelensis, T. v. kosswigi* and *T. v. graecus*); the latter two of which share ancestral behavioural and morphological features with *T. h. helveticus* (Raxworthy 1989a). The nominate subspecies, *T. v. vulgaris*, is geographically distributed over large parts of Europe and populations are sympatric with *T. h. helveticus* in parts of Britain and western Europe (Thorn 1968). The range of *T. h. helveticus* is more limited, but it extends further south to northern Spain and northern Portugal. There is only one other sub-species of *T. helveticus, T. h. sequeirai*, which occupies a very limited range in north-western Portugal (Thorn 1968).

Despite extensive geographical overlap, there have been few reports of naturally occuring hybrids between *T. v. vulgaris and T. h. helveticus* and only one of these has been substantiated unequivocally (Griffiths *et al.* 1987). Arntzen (1986b) failed to find hybrids between these two forms in

a specific investigation of hybridisation levels in syntopic French populations, suggesting that efficient reproductive isolating mechanisms must exist. In the remainder of this chapter I discuss the possible mechanisms operating, based on Mayr's (1963) model (Table 1.1). Unless otherwise indicated, use of the terms *T. vulgaris* and *T. helveticus* henceforth refers to the nominate subspecies.

PRE-MATING MECHANISMS.

Three groups of isolating mechanisms are included in this category. They may act independently to prevent interspecific crosses, or in combination.

Seasonal and habitat isolation.

Habitat exclusion of two sympatric species during the mating season forms a very effective barrier to interbreeding. The habitiat requirements of British amphibians have been discussed by Beebee (1981) and the factors which influence whether *T. vulgaris* or *T. helveticus* inhabit a particular pond have been investigated by Cooke and Frazer (1976), who found that *T. v. vulgaris* tended to prefer sites with high metal concentrations whilst *T. h. helveticus* tended to prefer sites with lower metal concentrations. They noted however, that both species were found in sites with similar physical characteristics. There have been many other reports of the two species occupying the same pond (e.g. Smith 1973, Harrison *et al.* 1983, Arntzen 1986b). Griffiths (1986, 1987) found that a high degree of overlap existed along feeding, microhabitat and seasonal niche dimensions, which suggests that the two populations in the pond he studied utlized common resources and furthermore, indicates that males and females of the two species have opportunities to meet one another.

Seasonal isolation may be important however, if the main courtship phases of the two species occur at different periods during the breeding season, or if mating takes place at different times of the day. The timing and duration of breeding migrations do not appear to be temporally separated (Harrison *et al.* 1983) and pairs of both species have been seen courting in the same pond at the same time of the day (Smith 1973). Courtship in *T. v. vulgaris* is largely confined to a brief period around dusk (Griffiths 1985), although some also occurs around dawn (Dolmen 1983). As similar studies have not yet been reported for *T. h. helveticus*, I carried out a brief investigation of the courtship activity times of this species at a pond at Castor Hanglands NNR, Cambs., which is also inhabited by a small population of *T. v. vulgaris*.

Method

Circuits of the pond were carried out at 20 minute intervals between 4.00am and 10.00pm GMT on 16 May 1984 and during each circuit, numbers of males and females and courting pairs of each species were recorded. Due to the low numbers of newts observed, scores per hour were pooled. Water temperature was noted hourly, and light intensity was recorded at 15 minute intervals using a light meter. *T. helveticus* was the main species of study but the data obtained for *T. vulgaris* have been included for comparison. Practical problems associated with observing fine detail accurately within a limited time period meant that scoring courtship encounter success was not possible on this occasion.

Results

Numbers of non-courting males and females and numbers of courting pairs per hour are shown in Figure 1.1, together with changing light intensity and water temperature. Despite large numbers of male *T. h. helveticus*

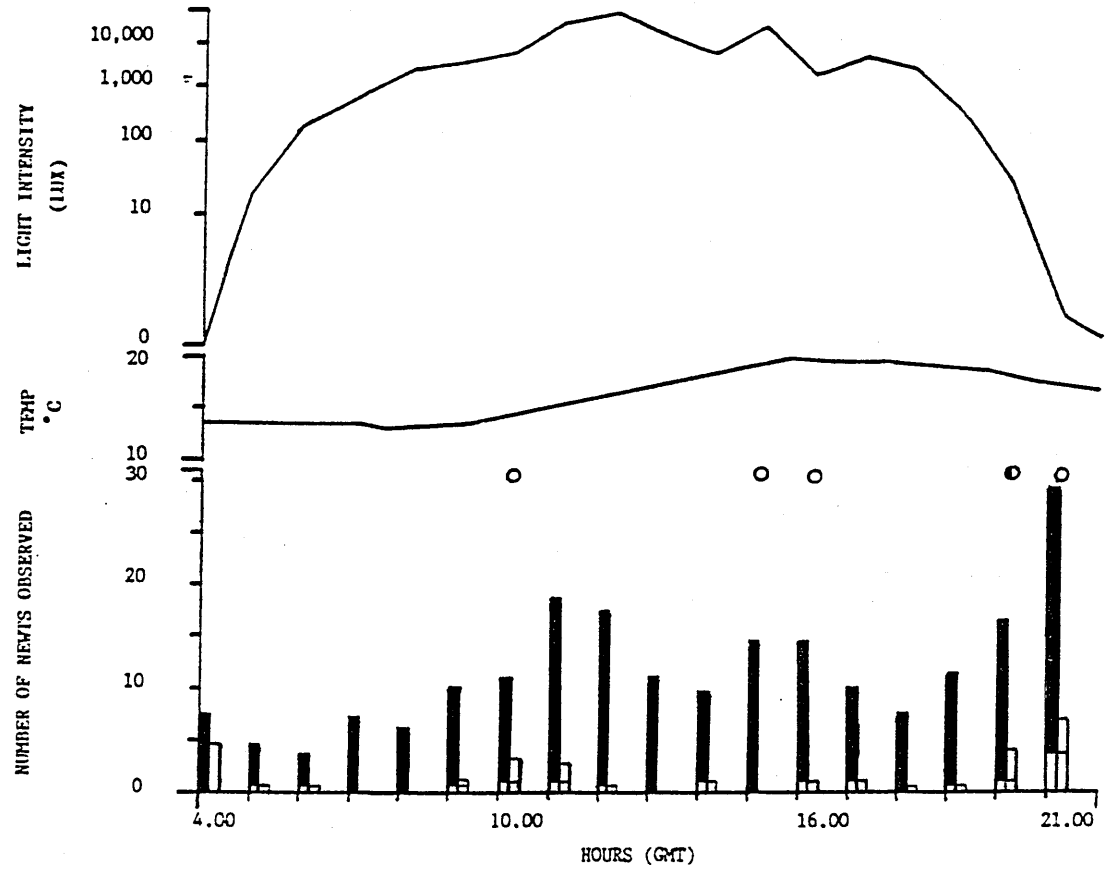


Figure 1.1 The timing of the courtship activity of *T. h. helveticus* in the field. Key to histograms: bold = male *T. h. helveticus*, open = female *T. h. helveticus*, red = courting pairs of *T. h. helveticus*. Circles: open = male *T. v. vulgaris*, half circle = male and female *T. v. vulgaris* (non-courting), red = courting pair of *T. v. vulgaris*.

being observed throughout the day, female *T. h. helveticus* availability was very low except at dawn, dusk and late morning. Courtship activity took place at several times of the day but was most noticeably absent at dawn when female availability was relatively high. The greatest number of courtship encounters were observed when light intensity was fading, but courtship activity does not appear to follow closely any trends associated with changes in water temperature. There were few *T. v. vulgaris* found during daylight hours, although courtship was observed between conspecifics at dusk. At no time was interspecific courtship observed.

Discussion

These preliminary data suggest that male *T. helveticus* will generally court females when they are available, but that dusk is an important time for courtship activity in this species. As only an 18-hour study was carried out, the importance of night-time courtship activity cannot be discounted and neither can the possibility of seasonal peaks in courtship activity. Verrell and Halliday (1985) suggested that there may be a particularly intense period of seasonal mating in May, shortly before the peak periods of synchronous ovulation observed in *T. vulgaris*; a recent field study by Verrell and McCabe (1988) carried out over the majority of the aquatic phase supports this. Unfortunately, neither a similar study on *T. helveticus*, nor a detailed quantitative field investigation comparing temporal differences in peaks of courtship activity in *T. vulgaris* and *T. helveticus* are available.

Due to the lack of information, I cannot draw firm conclusions relating to the absolute importance of seasonal and habitat isolation mechanisms except to say that in many ponds individual *T. vulgaris* and *T. helveticus* have the opportunity to both meet and mate during the breeding season.

Because of this, it would appear that seasonal and habitat incompatabilities are unlikely to be the primary mechanisms responsible for reproductive isolation in these two species.

Ethological isolating mechanisms.

Successful courtship is based on the production and reception of appropriate stimuli by the sexual partners. Tinbergen (1951) suggested that selectivity of mating behaviour is not dependent on one signal response but on a whole series. Inappropriate or inadequate stimuli produced on meeting or during courtship may act as an effective barrier to mating between heterospecific partners which prevents or reduces hybridisation. Mayr (1963) classified the components of ethological isolation in terms of the sensory modalities involved and emphasised that visual, auditory, tactile and chemical stimuli usually work in combination. In some species certain sensory modalities are better developed than others and only a limited number of the differences observed between species may be important ethological isolating mechanisms.

It is widely accepted that male *T. vulgaris* and *T. helveticus* differ considerably in their external morphology. Females of these two species however, are difficult to distinguish by humans, although a small number of distinguishing features have been reported (Veith & Dorr 1985). Halliday (1977a) recognised that morphological differences may be important during courtship and that visual cues may serve an isolating function. He suggested that the differences in epigamic characters between these two taxa evolved in sympatry together with differences in courtship through selection against hybrids (Halliday 1975). However, this hypothesis is rejected by Pecio and Rafinski (1985) who note that male *T.*

v. vulgaris occurs in regions of allopatry with typically developed epigamic characters.

The courtship behaviour of *T. v. vulgaris* has been the subject of extensive experimental analysis (e.g. Halliday 1974, 1975b, 1976; Teyssedre & Halliday 1986, Verrell 1983, 1984, 1986a, 1986b; Verrell & McCabe 1988) but that of *T. h. helveticus* is less well documented, although noteable exceptions include Halliday (1972) and Wambreuse and Bels (1984). Comparative accounts have highlighted differences in the early phases of courtship display which include the timing and frequency of display acts (Halliday 1977a, Wambreuse & Bels, 1984) and the angle (van Gelder 1977) and speed (Halliday 1975b, Raxworthy 1989a) of the fan display. The distinctive display differences may provide the female with visual cues of colour, contrast, pattern, form and movement, a tactile vibrational stimulus and additionally, a mechanism by which distinctive odours may be transferred (Halliday 1977a). Sniffing occurs frequently during courtship encounters in both species and male *T. vulgaris* exhibit a preference for larger conspecific females based on olfaction alone (Verrell 1986b). This evidence suggests that the capacity to respond to olfactory cues is well developed in male *T. vulgaris*.

Lantz (1947) argued that pre-mating isolating mechanisms may have evolved to prevent *T. v. vulgaris* from hybridising with *T. h. helveticus* as he found this cross difficult to achieve. A preliminary study carried out by Halliday (1977a) suggested that females will respond only to the display of conspecific males, possibly differentiating between conspecific and heterospecific males via several sensory modalities. There appear to be many factors which may function in part as ethological isolating mechanisms and which are worthy of further investigation. Before a full

appraisal of the importance of ethological mechanisms can take place, it will first be necessary to investigate possible differences in sensory stimuli between species and to test the response of potential partners to them. A quantitative study comparing the external morphology and courtship behaviour of *T. vulgaris* subspecies and *T. helveticus* has been carried out by Raxworthy (1989a), who suggested that the display of *T. v. vulgaris* places greater emphasis on visual stimulation, whilst that of *T. h. helveticus* places greater emphasis on olfactory stimulation. As yet the literature lacks reports of experiments investigating the importance of specific courtship behaviour and the possible sensory cues (visual, olfactory and tactile) involved.

POST-MATING MECHANISMS.

If pre-mating barriers fail, a second set of barriers may limit or prevent the success of hybridisation, although they do not prevent wastage of gametes. There are four categories in this group.

Mechanical isolation.

Newts of the genus *Triturus* differ from many sexually reproducing animal species by their mechanism of sperm transfer. Neither copulation nor amplexus occurs, but instead sperm transfer takes place by the deposition of a spermatophore which adheres on contact to the female's cloaca. Halliday (1974) called this process spermatophore pick-up. Consistent failure of a heterospecific spermatophore to adhere to the cloaca of a female would constitute an effective mechanical barrier. The spermatophores used by both male *T. vulgaris* and *T. helveticus* appear to be similar in size and morphology (Halliday 1977a) which suggests that they are unlikely to form a mechanical barrier. However, as this has not been empirically determined, I tested in the laboratory the success with which

heterospecific spermatophores would adhere to the cloaca of female *T. vulgaris* and *T. helveticus*.

Method

Ten females of each species were anaesthetised in m-aminobenzoate (MS222) and placed in a straitjacket harness (Halliday 1975b). When a courting heterospecific male had deposited a spermatophore, the cloaca of the female was brushed over the spermatophore in the natural manner. This was carried out with five females of each species for the first and second spermatophore deposited by a male. Success of pick-up was noted.

Results and Conclusion

Pick-up occurred for all of the female *T. vulgaris* presented with the first spermatophores deposited and four females presented with the second spermatophore deposited. The converse was found for female *T. helveticus*. The two spermatophores were missed probably because the edge and not the middle of the females' cloaca was brushed against the spermatophore. This is not an uncommon occurrence during conspecific encounters. The high incidence of heterospecific pick-up suggests that mechanical barriers can be discounted as a primary isolating mechanism.

Gametic mortality, zygote mortality and hybrid inferiority.

In *T. vulgaris* and *T. helveticus* fertilization is internal. Failure of the sperm to fertilize the ova after sperm transfer has taken place is recognised as gametic mortality. Wolterstorff and Freytag (1951) induced fertilization in *T. vulgaris* and *T. helveticus* by mixing excised eggs with sperm. Their results suggest that sperm can penetrate heterospecific egg membranes successfully, but one must be cautious in accepting this as evidence for the absence of gametic mortality in these species because

artificial external fertilization bypasses the possibility of sperm being damaged or killed by antigenic reactions in the genital tract or spermatheca of the female. Such an insemination reaction occurs in many species of *Drosophila* (Patterson & Stone 1952). Spurway and Callan (1960) found that a large proportion of eggs produced from *T. h. helveticus* x *T. vulgaris meridionalis* crosses by 'spontaneous insemination' failed to hatch. This suggests that gametic mortality may play some role as a post-mating isolation factor between these two sub-species. However, homologous data are not available for the nominate sub-species.

Development of the fertilized egg may cease at any stage between fertilization and adulthood, resulting in zygote mortality. Without detailed information it is difficult to ascertain whether the low hybrid egg hatching success observed by Spurway and Callan (1960) was due to gametic or early zygotic mortality, or to some other factor such as unsuitable rearing conditions.

Some hybrids may show hybrid inferiority, being phenotypically intermediate or abnormal and consequently less well adapted to their environment than either of the parental species. Spurway and Callan (1960) found that some of the adult male hybrids they studied showed evidence of hybrid vigour (particularly in terms of growth and early sexual maturity) but that their courtship behaviour was inadequate to stimulate a female. The male *T. helveticus* x *T. vulgaris* hybrid found by Griffiths *et al.* (1987) performed no more than sniffing behaviour toward females of both parental species despite repeated opportunities for courtship. This is again suggestive of hybrid inferiority.

There is currently little information available relating to the success of hybrid offspring produced from *T. vulgaris* and *T. helveticus* matings. To provide additional clues to the importance of the post-mating reproductive isolation mechanisms that may operate prior to adulthood, I carried out a comparison of the survival success of hybrid and purebred *T. vulgaris* and *T. helveticus* eggs and larvae to progressive life history stages, reared in a similar environment.

Method

Terrestrial phase female *T. vulgaris* and *T. helveticus* were captured by hand or pit-fall trapping to ensure that they had not mated that breeding season and aquatic male newts were captured at random using a dip-net. The animals were obtained from ponds at Newbridge-on-Wye, Milton Keynes and Oxford during March and April 1982. Prior to experimentation, they were housed in the laboratory in single-sex aquaria containing dechlorinated tap-water or pondwater at 10±2°C and water weeds. A light regimen approximating to the natural photoperiod was maintained and the animals were fed a mixed diet of chopped earthworms and aquatic invertebrates. The aquaria in which the female newts were housed were checked periodically for eggs or tadpoles which may have resulted from fertilization by sperm stored during the previous breeding season. None were found.

Once the females had become fully aquatic, ten females of each species were allowed to pick-up at least one spermatophore from a conspecific male during normal courtship. An attempt was made to invoke courtship display in male *T. vulgaris* and male *T. helveticus* by using anaesthestised harnessed heterospecific females whose behaviour was manipulated to mimic normal female responsive behaviour. Only those pairings between male *T.*

vulgaris and female *T. helveticus* were successful and led to spermatophore deposition. They led to six female *T. helveticus* being inseminated with at least one heterospecific spermatophore. After insemination, the females were placed in individual containers containing pond-water at 18.5°C and aquatic vegetation to lay their eggs.

Eggs were transferred daily with the portion of weed on which they were laid to aerated tanks (10 x 15 x 25 cms) and any eggs which subsequently suffered from fungal infection (probably *Saprolegnia)* were immediately removed. Tadpoles were transferred within 24 hours of hatching to aquaria (15 x 20 x 30 cms) containing up to 20 others. At all stages, eggs and larvae from different family groups were kept separated. The tadpoles were initially fed *Paramecium* and thereafter progressively larger zooplankton, particularly *Daphnia* and *Cyclops*. On metamorphosis, efts were maintained in 250ml evaporating dishes lined with damp tissue paper and covered with cellophane film which prevented both desiccation and escape. They were fed an *ad libitum* supply of *Drosophila*. The surviving efts were weighed on 17 October 1982, when they were between two and five months old. The number of eggs laid, success to hatching and metamorphosis and eft weight were noted and compared for the three groups. A failure in the laboratory cooling system produced heavy mortality the following month; those that survived (two purebred *T. vulgaris*) were released into the wild.

Results

The number of eggs laid per female varied greatly (minimum 0; maximum 255 for *T. helveticus* and 78 for female *T. vulgaris*). Egg hatching success was greatest for purebred *T. vulgaris* and poorest for hybrids (Table 1.2), whilst tadpole success to metamorphosis was similar in these two groups but lower for purebred *T. helveticus*. It was not known whether the large

proportion of eggs in each group that failed to develop were viable prior to fungal infection, or whether fungal invasion occurred after death. Fungal infection appeared to occur randomly, unlike tadpole mortality, which occured *en masse* in some aquaria, notably after an excess of prey had been given. Tadpole death in those instances was probably due to stress factors induced through overcrowding or the toxic products produced by decomposing zooplankton.

The proportion of larvae that survived as efts were similar for all groups. They either died at metamorphosis, when they failed to evacuate the water, or within a few weeks of metamorphosis. These latter animals were thin and appeared to pass few faeces, which suggests that eft mortality was probably also attributable in part to failure to feed successfully. A comparison of the weight of efts revealed no significant differences between the groups (Table 1.3).

	SUCCESS TO PROGRESSIVE LIFE-HISTORY STAGES				
	Eggs Laid	Eggs Hatched	Tadpoles Metamorphosed	Efts (to Oct)	Overall Survival
GROUP	n	%	%	%	%
T. vulgaris	203	78.3	35.2	73.2	20.2
T. helveticus	624	47.3	13.7	70.0	4.0
Hybrid	340	22.4	36.8	75.0	6.2

Table 1.2 Success and survival of larvae at each life history stage.

In contrast to the purebred groups which suffered the highest proportional mortality at the tadpole stage, hybrid loss was greatest at the egg stage. Overall, hybrid success was slightly better than that for *T. helveticus*, but poorer than that for *T. vulgaris*.

GROUP COMPARED	EFT WEIGHT		
	T. vulgaris	*T. helveticus*	Hybrid
Number of animals	41	25	21
Median	0.10g	0.10	0.10g
95% Confidence Limits	0.09-0.11g	0.09-0.11g	0.06-0.14g

Table 1.3 Purebred and hybrid eft weight. Mann-Whitney U Tests: Purebred forms - U=494, z=0.8394, P>0.05; *T. vulgaris* v hybrids - U=410, z=0.3049, P>0.05; *T. helveticus* v hybrids - U=234, z=0.6285, P>0.05.

Discussion

Many of the eggs that failed to develop successfully may not have been fertilized, but it is possible that the lower hybrid hatching success observed was partly a product of gametic or zygotic mortality. However, it is also possible that some of the eggs which were fertilized by heterospecific males produced zygotes which were less viable than purebred forms. This may have resulted in greater susceptibility to fungal infection.

The high incidence of tadpole mortality in all groups was probably a consequence of inexperience in rearing techniques, but there is sufficient evidence provided by the data for comparative success to later life history stages and eft weight to suggest that hybrid larvae which do hatch are subsequently as viable as purebred larvae, at least in the immediate post-metamorphic months when a ready supply of food is provided. Some efts weighed more than others, but it is more likely that the greater variance in weight of hybrids reflected differences in age more than genetic constitution.

Hybrid sterility.

Experimentally produced F1 hybrids between *T. v. meridionalis* and *T. helveticus* show varying degrees of anomalous spermatogenesis (Benazzi, 1957, Spurway & Callan 1960). Although the meiotic stages were not present at the time of capture of a *T. helvetcius x T. vulgaris* hybrid, apparently normal sperm bundles were observed in the testes (Griffiths *et al.* 1987). This natural hybrid was therefore probably fertile.

REPRODUCTIVE ISOLATION IN T. V. VULGARIS AND T. H. HELVETICUS - AN OVERVIEW.

The information presented in this chapter suggests that post-mating reproductive isolating mechanisms operating particularly during the earlier F1 hybrid larval stages may reduce the relative efficiency of heterospecific matings. However, some of the offspring from a natural heterospecific mating would be expected to metamorphose successfully, assuming that environmental conditions were favourable. One would therefore expect to find a number of hybrid larvae if heterospecifc matings occurred frequently in the wild. Arntzen's (1986b) investigation revealed no aquatic larval hybrids, which in combination with these data, strongly suggests that pre-mating barriers provide the primary isolating factor between *T. vulgaris* and *T. helveticus*.

As potential heterospecific mates have the opportunity to meet and there are no physical impediments to sperm exchange and uptake, it would appear that seasonal, habitat and mechanical isolation are not the primary pre-mating isolation mechanisms which operate. Much of the evidence suggests that sexual isolation is the most important and this has led me to concentrate on this area.

CHAPTER 2

A COMPARISON OF THE EXTERNAL MORPHOLOGY OF SMOOTH AND PALMATE NEWTS

Adults of the European species of *Triturus* show marked sexual dimorphism (Halliday 1975a, Raxworthy 1989a&b, Steward 1969, Thorn 1968) which, in terms of external morphology, is the most marked of all the Urodele genera (Halliday 1977a). Male *T. vulgaris* and *T. helveticus* differ from the females in many of their physical characteristics and they are easily identified during the breeding season by distinctive pigmentation patterns and physical features such as the size and shape of the dorsal crest; these have been used by taxonomists as definitive species characters. The females of these species are not as easily distinguished from one another by the untrained eye (Clifford 1986, Frazer 1983). However, they are generally separated by the unspotted throat of female *T. helveticus* (Frazer 1983, Thorn 1968, Veith & Dorr 1985).

A study of reproductive isolation would not be complete without a search for natural hybrids and intermediate morphology often provides the first and most useful clue to the genetic status of an individual for field workers. Creed (1964) thought that the marked similarity of *T. vulgaris* and *T. helveticus* larvae would make the recognition of juvenile hybrids by morphological means impossible. Hence, the low incidence of hybrid claims in the literature may be due in part to the failure of field workers to recognise natural hybrids in their larval stages of development when they would be at their most numerous.

THE MORPHOLOGY OF EFTS

Both Thorn (1968) and Steward (1969) reported that the aquatic larvae of *T. vulgaris* and *T. helveticus* may be distinguished by comparing the

distance between the nostril and the eye with that of the horizontal diameter of the eye, but this method has been found unreliable by J. Harrison (pers. comm.) and it would presumably also be unreliable for distinguishing hybrid larvae. No diagnostic morphological features have been reported for efts of these two species in the literature. However some differences appeared to exist in the vertebral stripe characteristics of purebred efts (Plate 1 & 2) reared during an earlier investigation (Chapter 1). In this section purebred eft stripe characteristics and belly colours are described and compared to those of hybrids, to determine whether sufficient differences exist between the groups to permit a feasible search for hybrid efts based on morphological methods.

Method

The purebred and hybrid efts bred in the manner described on page 32 were used as subjects in this study. Purebred efts were placed at random in numbered phials and presented blind to a pilot subject who described for each eft, the intensity of pigment distribution of the vertebral stripe, the vertebral stripe colour and the belly colour according to the categories given in Table 2.1, and then allocated them to species according to the following preliminary guidelines: *T. vulgaris* - Yellow-ish uniformly pigmented stripe; *T. helveticus* - Orange-ish stripe most intensely pigmented anteriorally. During the test it became apparent that the position of the posterior end point of the stripe may also be used as a distinguishing feature. The test was repeated by four subjects using revised guidelines; these subjects further suggested that the anterior starting point of the stripe also differed between species. To test the suitability of the latter differences for distinguishing species, six subjects described the starting position of the stripe according to the categories listed in Table 2.1.

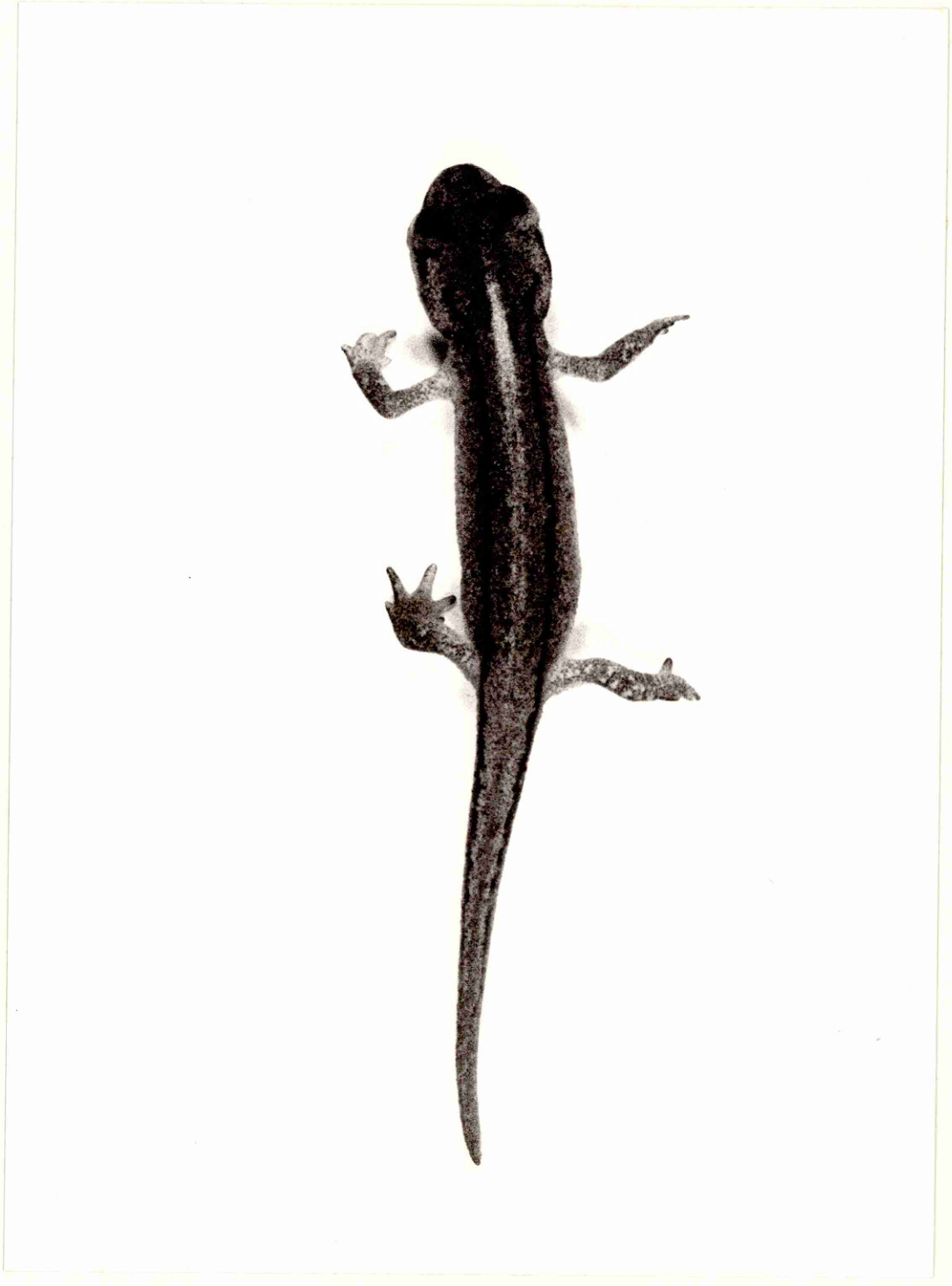


Plate 1 A typical *T. v. vulgaris* eft.

Plate 2 A typical *T. h. helveticus* eft.

CHARACTER	DESCRIPTION	RANK
Anterior Stripe Starting Position	No stripe	0
	Central region of the head	1
	Base of the head	2
	Neck	3
	Neck/Trunk interface	4
	Trunk	5
Posterior Stripe Ending Position	No stripe	0
	Trunk	1
	Pelvic Girdle	2
	Tail	3
Stripe Pigment Distribution	No stripe	0
	Most intense at anterior end	1
	Uniformly distributed	2
	Other	3
Stripe Colour	No stripe	0
	Yellow	1
	Pale orange	2
	Orange	3
	Deep orange	4
Belly Colour	Yellow	1
	Pale orange	2
	Orange	3
	Deep orange	4

Table 2.1 Ranked descriptive categories of eft morphological characters.

Although the level of accuracy in distinguishing the forms was high (up to 98% accuracy was achieved), homogeneity in the use of descriptive terms between observers when describing the same animal was generally poor (Table 2.2) which indicated that each subject used a different descriptive term for what they saw. To enable guidelines to be compiled for the morphology of purebred forms against which potential hybrids could be compared, each descriptive term for a particular feature was ranked (Table 2.1) and these scores were added to obtain a group score per eft for each character. Differences between group scores for each form were tested for significance using two-tailed Mann-Whitney U Tests. Guidelines for distinguishing purebred efts are based on the descriptive

term approximating to the median of the group scores corrected for the number of subjects.

CHARACTER	N. of observers	PERCENTAGE HOMOGENEITY *T. vulgaris*	*T. helveticus*
Anterior start position	6	92.7	16.0
Posterior end position	4	7.3	57.7
Pigment distribution	5	36.6	69.2
Stripe colour	5	nil	7.3
Belly colour	5	9.8	3.8

Table 2.2 Homogeneity in the use of morphological terms. Number of animals in each group - *T. v. vulgaris*, n=41 for all characters; *T. h. helveticus*, n=25 for anterior start position; n=26 all other characters.

By using the same method as that described earlier, hybrid and purebred efts were presented blind to one subject only (JMR), to maintain homogeneity of description. This subject described the five characters listed in Table 2.1 for each animal, but did not identify the forms. Median descriptions for the purebred and hybrid cohorts were obtained.

Results and conclusions

Significant differences were found betwen purebred efts in all four of the vertebral stripe characteristics, but none were observed for belly colouration (Table 2.3). Subjects showed least homogeneity when describing stripe colour, which suggests that this should not be used as a primary distinguishing feature. The following general descriptions are offered as guidelines for distinguishing purebred efts:-

T. vulgaris. The anterior end of the vertebral stripe begins high on the head and usually ends near to, or at the pelvic girdle. It is more intensely pigmented at the anterior end and becomes weaker towards the

posterior end. The stripe colour varies from yellow to deep orange but it is most commonly pale orange.

T. helveticus. The anterior end of the vertebral stripe commences at the base of the head or in the neck region and usually extends beyond the pelvic girdle. It is uniformly pigmented along its length and tends to be orange in colour, but the shade may vary from pale to deep orange.

When data such as these are obtained from a relatively limited laboratory reared cohort, their value when applied to a field environment must be questioned. However, using electrophoresis as an independent means for confirming species, R. Griffiths (pers. comm.) tested and found this morphological technique to be highly reliable for distinguishing wild efts.

A COMPARISON OF PUREBRED EFT MORPHOLOGY					
	n		Mann-Whitney U Tests		
CHARACTER	*T. v.*	*T. h.*	U	z	P
Anterior start position	41	25	25.0	6.4441	<0.001
Posterior end position	41	26	63.5	6.0408	<0.001
Pigment distribution	41	26	30.5	6.4654	<0.001
Stripe colour	41	26	111.5	5.4232	<0.001
Belly colour	41	26	498.0	0.4503	>0.05

Table 2.3 A comparison of *T. v. vulgaris* and *T. h. helveticus* eft morphology based on group scores.

Overall, the hybrid efts were most similar to the maternal species, *T. helveticus* (Table 2.4). Over half (52%) possessed some vertebral stripe characters associated with both purebred forms: all of these had stripes which began high on the head, were uniform in pigmentation and which ended at the pelvic girdle or on the tail. The colour of the stripes was either orange or pale orange. All the characters of the remaining hybrid efts had characters were typical of either *T. vulgaris* (19%), or *T.*

helveticus (29%). On the basis of these data, the earlier lack of homogeneity observed and because no unique morphological features emerged by which they could be distinguished, it is clear that a search for hybrids based on eft stripe morphology would give misleading results.

CHARACTER	AVERAGE MORPHOLOGY OF FORM *T. vulgaris*	*T. helveticus*	Hybrid
Anterior stripe start position	Head	Base of head	Head
Posterior stripe end position	Pelvic girdle	Tail	Tail
Stripe pigment distribution	Most heavily pigmented anteriorally	Uniform	Uniform
Stripe colour	Pale orange	Orange	Orange
Belly colour	Pale orange	Pale orange	Pale orange

Table 2.4 A general description of purebred and hybrid eft morphology. NB: The median (group score/n. observers) for each character has been allocated the descriptive term to which it most closely approximates for the purebred forms; median hybrid descriptions are based on the concepts of one observer.

THE COMPARATIVE MORPHOLOGY OF ADULT *T. VULGARIS* AND *T. HELVETICUS*

Adult hybrids between the subspecies of *T. vulgaris* and *T. helveticus* have intermediate morphology (Griffiths *et al.* 1987, Spurway & Callan 1960, Wolterstorf & Freytag 1951), but a search for hybrids is only feasible when a sound knowledge of the morphology of the parental species is available for comparison. Most reports of the morphology of adult male and female *T. vulgaris* and *T. helveticus* have either been restricted to qualitative descriptions, or to quantitative accounts of a limited number of characters. Here, I provide both a comprehensive quantitative comparison of the external morphology of the two species and further

evidence that many morphological differences exist which may provide newts with cues on which they may discriminate. Due to visual predelictions, humans may consider certain characters more important in species discrimination than others and so a multivariate mathematical approach, based on a comprehensive data set, has been used in conjunction with other statistical tests for discriminating between the four groups and to determine which are the most reliable distinguishing characters.

Method

During the months of March to June in 1982 and 1983 samples of newts were collected on land and from the water at the sites listed in Table 2.5. A total of 126 male *T. vulgaris*, 130 female *T. helveticus*, 64 male *T. helveticus* and 85 female *T. helveticus* were taken. To ensure that each animal was used only once during the study, individuals were marked by removing one of the forelimb digits and the pattern of distinctive belly or throat markings were noted for reference. Within 24 hours of capture the newts were returned to the laboratory and measured under 'Varilux' fluorescent tubes which emit light at the same wavelengths found in the natural environment. After anaesthesia in a weak solution of m-aminobenzoate (MS222) a catalogue of records was established for each animal, later transcribed on to DEC computer file, which, in addition to morphological data relating to 24 physical characters, also included a unique identification number, details of sex, date and place of capture, a note on whether the animal was terrestrial or aquatic and comments on any unusual features.

Qualitative general descriptions of typical males and females of each species during the aquatic phase were made making comparisons between the

groups where appropriate. A note was also made of the differences between aquatic and terrestrial phase newts.

FORM	SITE	PHASE AND MONTH OF CAPTURE Terrestrial March	Aquatic March	Aquatic April	Aquatic May	Aquatic June
Male *T. vulgaris*	LC	4	0	18	21	12
	OX	0	20	24	0	1
	MK	26	0	0	0	0
Female *T. vulgaris*	LC	7	1	23	24	6
	CH	0	1	1	0	0
	NE	3	0	0	0	0
	OX	0	2	10	0	14
	MK	34	0	0	0	0
	WO	0	0	4	0	0
Male *T. helveticus*	LC	1	0	4	23	1
	CH	0	18	12	0	0
	LA	5	0	0	0	0
Female *T. helveticus*	LC	2	0	5	11	12
	CH	0	8	12	0	10
	LA	10	0	0	0	0
	NE	15	0	0	0	0

Table 2.5 Site and month of capture of terrrestrial and aquatic newts.
Key to sites: LC=Littleworth Common, Bucks.; OX=Oxford, Oxon.; MK=Milton Keynes, Bucks.; NE=Newbridge-on-Wye, Powys.; WO=Woburn, Bucks; CH=Castor Hanglands NNR, Cambs.; LA=Lancaster, Lancs.

A list of the physical features studied is presented in Table 2.6 and the positions on the body from which they were taken is illustrated in Figure 2.1. After surface-drying with tissue, weight (WT) was obtained using an electronic Mettler Pan Balance and girth (GI) at maximum point was measured using a graduated circlet made of $1mm^2$ graph paper reinforced with sellotape. Other measurements (SV, TL, HW, CH, TD, FL, LL, AL, ID, HL, WEB, END, IDL) were made with a metric precision steel rule; units of measure and levels of accuracy are shown in Table 2.6. The length of the tail (TAL) was calculated as total length (TL) minus snout-vent length

(SV). Counts were made of the number of belly (BSN) and throat spots (TSN). A spot that crossed the ventral neck crease was defined as belonging to the region (i. e. throat or belly) in which it occupied the greatest area. The development of the fine membrane originating at the side of the upper part of the mouth (MD), the density of spot pigmentation (DSP) and the size (BSS) of belly spots and in male newts only, the shape of the dorsal crest (CS) were described using the ranking systems shown in Figure 2.2. Finally, as standard colour charts contained too few shades, colours (BEC, BOC) were compared to the 'Dulux Matchmaker' range of paint shades: those found are presented in the Appendices.

The data collected for the 24 characters fell into one of three statistical groups. The majority (18) were ratio, four were nominal (BSS, DSP, MD, CS) and the remaining two (BEC, BOC), ordinal. Consequently, non-parametric statistics have been used to maintain consistency of presentation and data analysis.

All characters were compared between the following groups - male *v* female *T. vulgaris*, male *v* female *T. helveticus*, male *T. vulgaris* *v* male *T. helveticus* and female *T. vulgaris* *v* female *T. helveticus* using two-tailed Mann-Whitney U Tests, with the exception of colours which could not be realistically assigned a meaningful rank score. Statistical analyses were carried out on a DEC computer using standard commands from SPSS (Nie *et al.* 1975) compiled to correct for ties and missing data points. Those characters which showed the greatest and those which showed no separation were judged the most and least suitable, respectively, for distinguishing the groups.

A morphometric discriminant function analysis was carried out using the DISCRIMINANT command from SPSSX (Version 3) for the aquatic phase animals, firstly to visually depict the degree of separation of the four groups based on a combination of each of the individual newts' physical characters and secondly, to determine the accuracy of distinguishing newts by this mathematical method. Characters which were omitted from this analyses were colours (BOC, BEC), which could not be assigned a meaningful rank score, tail length (TAL), which failed the discriminant tolerance test (i.e. the variance of this character was too similar to others) and number of head ridges (NHR), which when omitted produced more accurate results.

CHARACTER	ABBREVIATION
Weight #	WT
Girth *	GI
Snout-vent length *	SV
Total length excluding filament *	TL
Head width 1mm behind eyes *	HW
Crest height *	CH
Tail depth at deepest point *	TD
Filament length **	FIL
Tibia/fibula length *	AL
Femur length *	LL
Internostril distance *	ID
Head length *	HL
Webbing width *	WEB
Eye-nostril distance *	END
Intermedium (wrist) - digit length *	IDL
Number of head ridges	NHR
Number of belly spots	BSN
Number of throat spots	TSN
Upper mandible membrane development ##	MD
Density of spot pigmentation ##	DSP
Size of belly spots ##	BSS
Crest shape ##	CS
Belly colour ***	BEC
Body colour ***	BOC
Tail length §	TAL

Table 2.6 The physical characters recorded. Key: * = Measured to nearest 0.5mm; ** = Measured to nearest 0.5mm unless measurement is less than 1.0mm, when character was measured to nearest 0.25mm; *** = See colours in Appendices; # = Measured to nearest 0.01g; ## = See Figure 2.2 for ranking system; § = Calculated from TL - SV.

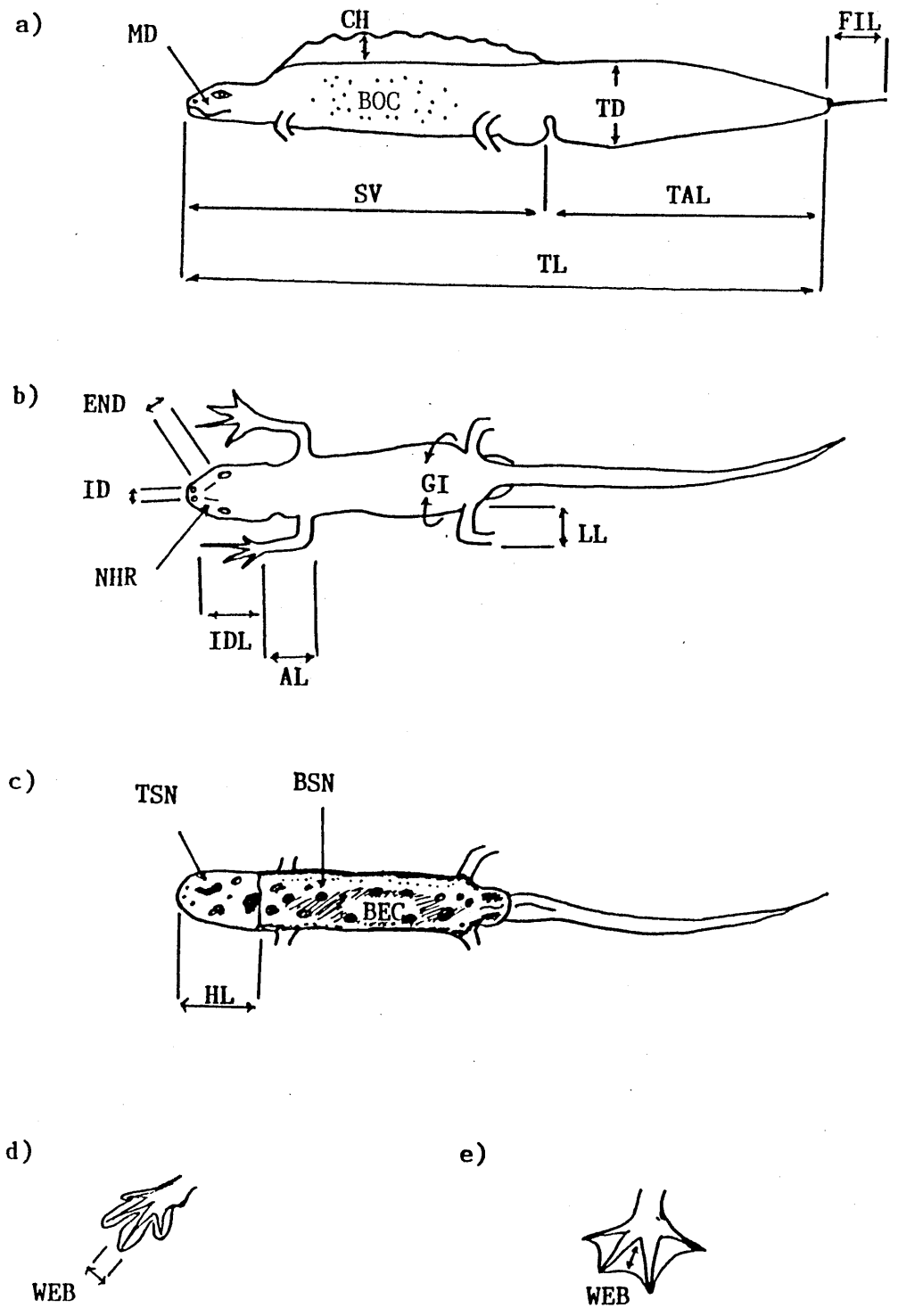


Figure 2.1 Position of physical characters recorded. Abbreviations relate to the descriptions listed in Table 2.5: a) Lateral view; b) Dorsal view; c) Ventral view; d) *T. vulgaris* webbing; e) *T. helveticus* webbing

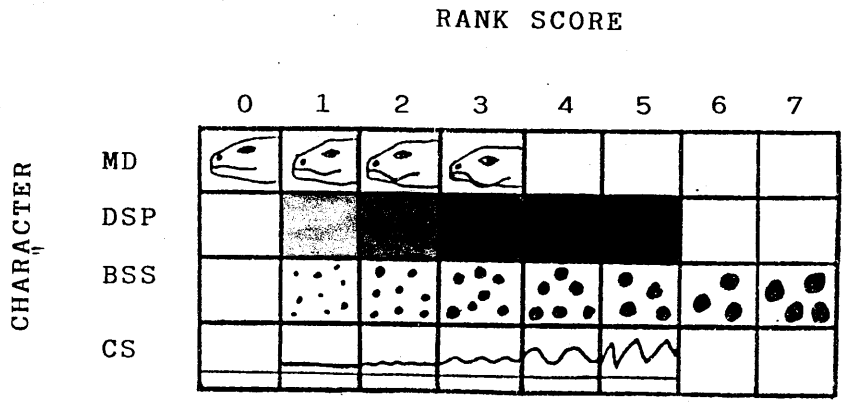


Figure 2.2 Ranking system for ordinal characters. Where for *Mandible Membrane Development* (MD) 0=absent, 1=below mouth, 2=extending over lower jaw, 3=extending below lower jaw; *Density of belly spot pigmentation* (DSP) 0=spots absent, 1=faint, 2=pale, 3=medium, 4=dense, 5=very dense; *Size of belly spots* (BSS) 0=spots absent, 1=speckles, 2=small, 3=small-medium, 4=medium, 5=medium-large, 6=large, 7=very large; *Shape of dorsal crest* (CS) 0=crest absent, 1=smooth, 2=slightly denticulated, 3=denticulated, 4=heavily denticulated, 5=serrated.

Results

A general description of aquatic phase newts.

Male *T. vulgaris* are large in size with a slim, rounded body which is roughly equal in length to the tail. The forelimbs and forelimb digits are long and the hindlimb digits are each fringed with a fine membrane. Starting in the neck region and running along the vertebral column is a denticulated dorsal crest which is continuous with the deep tail. These denticulations become less distinct toward the distal portion of the tail, which gradually tapers to a fine tip. The flanks are mid to light brown and the bulbous cloaca is dark brown or black. These regions contrast with the central region of the belly which is usually orange with a faint yellow-brown periphery. This latter colouration is due to a layer of

pigmentation which also extends onto the throat. The pigmentation pattern of the male's tail is quite distinctive - mainly light brown in colour, but adjacent to the cloaca at the base of the tail there are horizontal orange, red and silvery-blue bands. Large dark spots are interspersed across the throat, belly, dorsal crest, flanks and tail with closely-grouped smaller spots on the dorsal surface of the head. On each side of the head are two dark stripes (running across and below the eye from snout to neck) and on the dorsal surface of the head are two fine ridges, located behind the snout. There is also a pair of fine transparent upper mandible membranes which extend over the lower jaw.

Female ***T. vulgaris*** are similar in size to conspecific males but they have a plumper, rounded body, with a snout-vent length slightly shorter than the tail length. They lack both a dorsal crest and hind-limb webbing, but their tail, which is smooth-edged and not as deep as the males', also tapers gradually to the tip. Their flank and tail colour is generally light brown and there is a fine horizontal orange stripe running along the base of the tail adjacent to the small, light-brown cloaca. The belly is pale orange and it is surrounded by a faint yellow-brown periphery which also extends onto the throat. Both the belly and throat are speckled with a large number of small brown spots, but on the flanks and tail they are not as distinctive and frequently give a mottled appearance. Across each eye is sometimes found a single horizontal pale brown stripe and on the dorsal surface of the head are two faint ridges. The transparent upper mandible membrane may extend over the lower jaw. Females caught at the start of the aquatic phase frequently possess a yellow-orange dorsal stripe similar to that described earlier for efts of this species.

Male *T. helveticus* are the smallest of the four forms studied. They have a short, slim trunk, slightly concave below each of the pronounced dorso-lateral folds and a low smooth dorsal crest which is not continuous with the tail. The tail, which is also undenticulated, is deep and at its truncated distal end is a fine tapering tail filament. The tail, excluding the filament, is shorter than the length of the head and body. The digits of the forelimbs are relatively long and the hindlimb digits are fully proximally webbed; this webbing is sooty in colour. In males with well developed secondary sexual characteristics, a white heel spur is present on the hindlimbs. Two faint ridges are found behind the snout on the dorsal surface of the head and a black eye stripe runs from snout to neck. The upper mandible membranes are poorly developed. Male *T. helveticus* have very distinctive colouration and patterning. They have green-brown marbling on the dorsal surfaces and flanks, whilst the central portion of the belly ranges in shade from pale straw to orange and it is speckled with a small number of dark spots. The periphery of the belly is milky-white and the throat is unspotted and pale pink or white in colour. On the tail there is a central deep red region which is bordered on either side by a row of large dark spots which diminish in size towards the tail filament.

Female *T. helveticus* are larger and heavier than conspecific males, but are generally a little smaller in size than female *T. vulgaris*. They have a broad head and a rounded, plump body with a snout-vent length that is longer than the tail. They lack the dorsal crest, hind-limb webbing, dorso-lateral folds, marbled body pigmentation pattern, white hindlimb spurs and truncated tail of conspecific males; 28% of females measured of this species had a vestigal tail filament protruding from their gradually tapering tail tip. The tail is proportionally not as deep as in males of

the same species, but the tail colouring and pigmentation patterns are similar. In addition, the throat and belly colours and spot distribution pattern is like that of conspecific males, but a slightly greater number of small, pale spots are present on the belly. As on the throat, the head and flanks are unspotted. The latter two range in colour from light brown to pale olive and the cloaca is light brown. The pair of transparent upper mandible membranes is well-developed.

A comparison with terrestrial phase newts.

Terrestrial newts from all four groups differ markedly from aquatic phase animals and they are much more difficult to distinguish. They have rough, dry skin and muted, cryptic lateral and dorsal spot patterning and coloring, which contrasts markedly with the slimy body and conspicuous coloring and distinctive patterning of aquatic phase animals. The ventral colours however, retain their brightness. Integumental features in male newts, such as hind limb webbing are usually absent, although some vestigial epigamic characteristics may be retained, particularly the tail filament in male *T. helveticus*. In both males and females, the upper mandible membranes are absent.

Quantitative details of aquatic newts.

The data (Table 2.7 - 2.10) indicate that there is a great deal of overlap in the range of measurements and colour shades for all groups, excepting those features which are not found in that group e.g. tail filament in *T. vulgaris*, hind limb webbing in female newts. The range of body colours of male *T. vulgaris*, female *T. vulgaris*, male *T. helveticus* and female *T. helveticus* was from mid to dark brown, light to dark brown, pale olive to dark brown and light brown to olive, respectively. The respective ranges of belly shades varied from mid to deep orange, pale to mid orange, very pale yellow to mid orange and pale yellow to mid orange.

CHARACTER	GROUP MEASUREMENTS Male *T. vulgaris* median	range	Female *T. vulgaris* median	range
Weight (g)	2.10	0.98 - 3.10	1.89	1.11 - 3.54
Girth (mm)	22.1	18.0 - 30.0	23.2	19.0 - 31.0
Snout-vent length (mm)	42.7	33.0 - 50.5	41.7	33.5 - 51.5
Total length (mm)	84.9	65.0 - 100.5	78.7	65.5 - 97.5
Head width (mm)	6.8	5.0 - 9.0	6.8	5.5 - 8.5
Crest Height (mm)	1.8	0.5 - 4.5	-	-
Tail depth (mm)	10.9	6.0 - 18.0	6.7	3.5 - 9.5
Filament length (mm)	-	-	-	-
Tibia/fibula length (mm)	4.3	3.5 - 5.5	4.0	3.0 - 5.0
Femur length (mm)	5.6	4.5 - 7.0	5.5	4.0 - 7.0
Int.-nost. distance (mm)	1.9	1.5 - 3.0	1.9	1.5 - 2.5
Head length (mm)	10.2	8.5 - 12.0	9.9	8.0 - 12.5
Webbing width (mm)	0.8	0 - 2.5	-	-
Eye-nost. distance (mm)	2.4	1.5 - 3.5	2.1	1.5 - 3.0
Int.-digit length (mm)	6.3	4.0 - 9.0	5.5	4.0 - 7.0
Number of belly spots	25.4	14 - 47	46.0	0 - 100
Number of throat spots	13.2	1 - 32	21.2	0 - 65
Tail length (mm)	41.2	29.5 - 55.0	37.5	21.5 - 51.0

Table 2.7 Median and range of aquatic *T. v. vulgaris* characteristics.

CHARACTER	GROUP MEASUREMENTS Male *T. helveticus* median	range	Female *T. helveticus* median	range
Weight (g)	1.37	1.11 - 3.53	1.91	1.13 - 2.72
Girth (mm)	21.7	18.0 - 25.0	23.9	19.0 - 31.0
Snout-vent length (mm)	35.6	31.5 - 39.0	39.8	36.0 - 49.5
Total length (mm)	64.2	56.5 - 72.0	73.2	66.5 - 82.5
Head width (mm)	6.2	5.5 - 8.0	7.0	6.0 - 8.0
Crest height (mm)	0.5	0.0 - 1.5	-	-
Tail depth (mm)	8.8	5.0 - 11.5	6.6	5.0 - 8.0
Filament length (mm)	3.9	1.0 - 7.0	0.20	0.0 - 1.5
Tibia/fibula length (mm)	4.1	3.5 - 5.0	4.1	3.5 - 5.0
Femur length (mm)	5.2	4.0 - 6.5	5.8	4.5 - 7.0
Int.-nost. distance (mm)	1.7	1.5 - 2.0	1.8	1.5 - 2.0
Head length (mm)	8.9	6.5 - 10.5	9.9	8.5 - 11.5
Webbing width (mm)	1.95	0.25 - 3.50	-	-
Eye-nost. distance (mm)	2.2	2.0 - 3.0	2.2	1.5 - 4.5
Int.-digit length (mm)	5.6	4.0 - 7.0	1.8	1.5 - 2.0
Number of belly spots	16	0 - 35	12.8	0 - 48
Number of throat spots	0	0 - 3	0	0 - 1
Tail length (mm)	28.6	23.0 - 34.0	34.1	23.5 - 41.5

Table 2.8 Median and range of aquatic *T. h. helveticus* characteristics.

CHARACTER	*T. vulgaris* male m	male range	*T. vulgaris* female m	female range	*T. helveticus* male m	male range	*T. helveticus* female m	female range
Membrane d/ment	2	0-2	2	0-3	1	0-2	2	1-2
Den. spot pig.	4	2-5	3	0-4	3	0-4	1	0-3
Belly spot size	5	1-7	2	0-5	2	0-4	2	0-6
Crest shape	3	2-4	-	-	1	0-1	-	-

Table 2.9 Mode and range of (ordinal) morphological characters. The ranking system corresponding to the integers used for modes and ranges is illustrated and described in Figure 2.2.

Distinguishing features.

Many of the physical features showed highly significant differences between the forms. The most highly separated (i.e. $z<-6.000$; $P<0.001$) characters for each of the pairs compared are listed in Table 2.11 together with those that showed no significant separation (i.e. $P>0.05$) and these form a general guide to the most and least suitable distinguishing characters, respectively. Integumental characters, limb measurements and spot characteristics were amongst the most important groups of features, particularly the former group when distinguishing females from males and the latter group when distinguishing females of the two species.

Discriminating between the newt groups.

The discriminant function analysis successfully classified (Table 2.12) and separated (Figure 2.3) the majority of individuals comprising all four newt forms captured in aquatic phase. Hence male and female *T. vulgaris* and *T. helveticus* are mathematically distinguishable with accuracy. However, a slight degree of overlap was observed between the female groups; 5.8% of female *T. vulgaris* were mis-classified as female

COLOUR# (Approx)	DULUX COLOUR CODE*	GROUP male *T. vul*	female *T. vul*	male *T. helv*	female *T. helv*
BELLY COLOURS:					
Deep	669	2	-	-	-
orange	737	19	-	-	-
	741	24	8	-	-
	646	30	22	5	5
Orange	567	12	49	9	9
	587	1	1	-	-
	708	4	2	-	3
Pale	897	2	1	2	3
orange	820	4	8	10	28
	734	-	1	3	-
	521	-	-	2	-
Yellow	753	2	5	22	19
	576	-	-	12	7
	837	-	2	17	16
	619	-	-	5	3
Pale	778	-	-	2	5
yellow	723	-	-	2	-
	869	-	-	9	2
BODY COLOURS:					
	595	2	-	-	-
	724	-	-	-	2
Green	920	-	1	-	2
	678	-	-	3	-
	670	-	5	24	24
	552	3	29	36	33
	629	-	-	5	5
	744	-	-	2	-
Green-brown	739	-	-	9	2
	505	-	-	2	-
	618	-	-	-	2
	775	-	1	2	-
	523	1	2	2	2
	871	-	6	2	5
Brown	661	-	5	-	3
	574	43	43	14	19
	696	20	4	-	-
	892	14	1	-	-
	571	4	-	-	-
	551	3	1	-	-
Light	572	2	-	-	-
brown	784	2	-	-	-
	772	6	2	-	2

Table 2.10 Belly and body colours. # = Shades named according to my personal colour concepts. * = Code corresponds to those presented in Appendices I & II.

	GROUP A	GROUP B	GROUP C	GROUP D
Most Suitable Distinguishing Characters	CH TAL CS IDL TD DSP WEB BSS IDL BSN TL SV	CH TAL CS WT FIL HW WEB HL TD DSP TL BSS	CH TL CS TAL WEB SV FIL DSP TD BSS WT BSN HL TSN	FIL BSN TSN DSP
Least Suitable Distinguishing Characters	SV ID NHR HW MD	NHR ID END AL	MD	NHR WT ID END HL IDL TD MD

Table 2.11 The most and least suitable characters for distinguishing aquatic phase male and female *T. v. vulgaris* and *T. h. helveticus*. Forms compared: GROUP A) Male *T. vulgaris* v female *T. vulgaris*; B) Male *T. helveticus* v female *T. helveticus*; C) Male *T. vulgaris* v male *T. helveticus*; D) Female *T. vulgaris* v female *T. helveticus*. Abbreviations correspond to characters listed in Table 2.6.

		PREDICTED GROUP MEMBERSHIP			
FORMS	n cases	Male *T. vulgaris*	Male *T. helveticus*	Female *T. vulgaris*	Female *T. helveticus*
Male *T. vulgaris*	92	92 100.0%	0 0.0%	0 0.0%	0 0.0%
Male *T. helveticus*	57	0 0.0%	56 98.2%	0 0.0%	1 1.8%
Female *T. vulgaris*	86	0 0.0%	0 0.0%	81 94.2%	5 5.8%
Female *T. helveticus*	57	0 0.0%	0 0.0%	0 0.0%	57 100.0%

Table 2.12 Accuracy of classification for aquatic phase newts.
Percentage of grouped cases correctly identified: 97.95%.

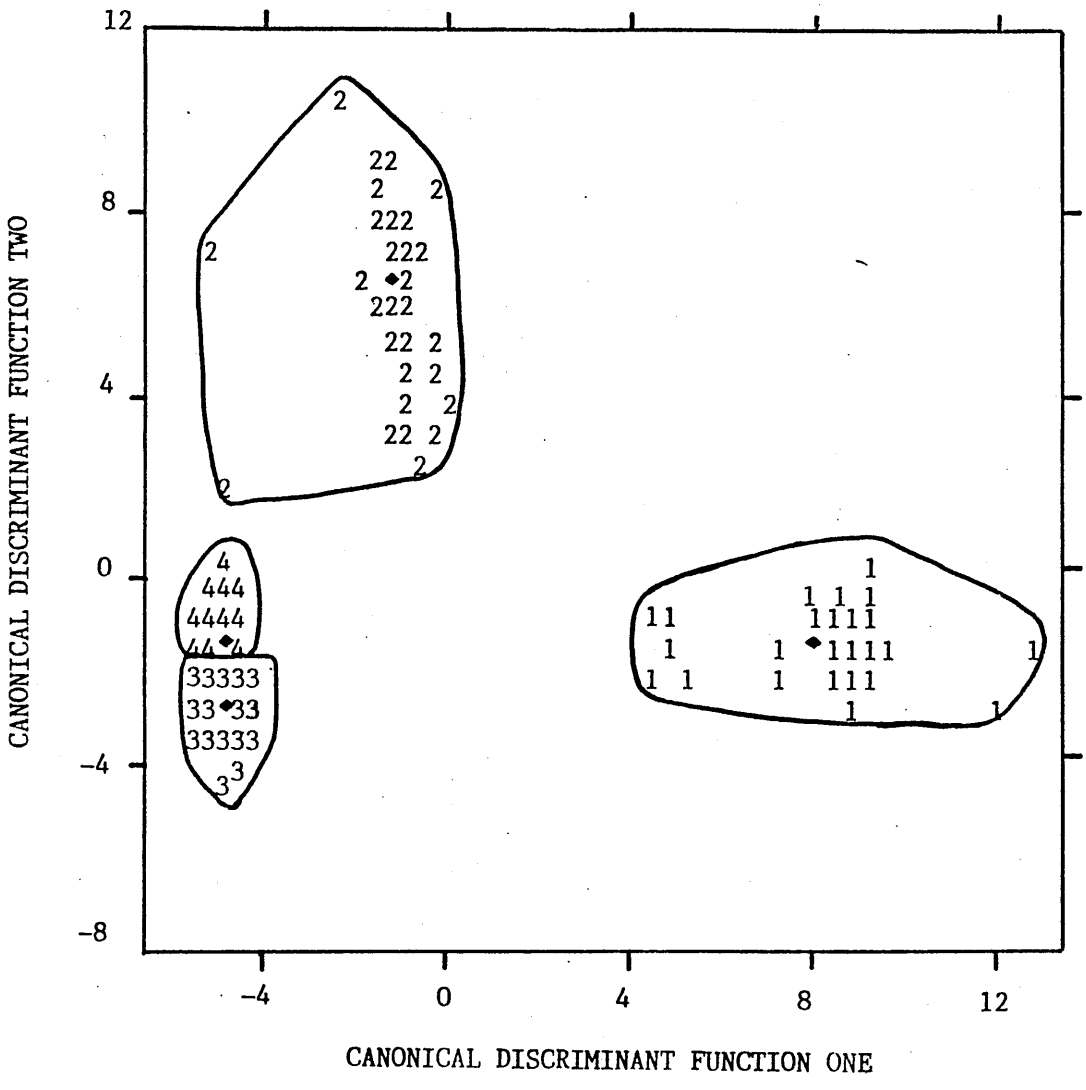


Figure 2.3 Discriminant function analysis of aquatic newts. Key to points: 1 = Male *T. v. vulgaris*; 2 = Male *T. h. helveticus*; 3 = Female *T. v. vulgaris*; 4 = female *T. h. helveticus*; Canonical discriminant functions evaluated at group mean (group centroid ◆):

	Function One	*Function Two*	*Function Three*
Male *T . v. vul*	8.16447	-1.13416	-0.09354
Male *T. h. hel*	-1.14390	6.53796	0.41952
Female *T. v. vul*	-4.72529	-2.45152	1.46296
Female *T. h. hel*	-4.90445	-1.00860	-2.47582

Functions (F):

F	*Eigen-value*	*% Var*	*Cumul, %*	*Canonical Correlation*		*After Funct,*	*Wilks' Lambda*	χ^2	*df*	*Sig*
					;	0	0,0008581	1966,4	63	0,0000
1	32,98080	72	72	0,9851759	;	1	0,9851759	984,49	40	0,0000
2	10,86683	24	96	0,9569386	;	2	0,3460327	295,55	19	0,0000
3	1,88990	4	100	0,8086824	;					

CHARACTER	FUNCTION 1	2	3
CS	0.92418*	0.08065	-0.01249
CH	0.28632*	0.00235	-0.01216
TD	0.20696*	0.06326	0.05296
BSS	0.15926*	-0.09582	-0.15238
IDL	0.09667*	-0.01564	0.03506
END	0.05346*	-0.00395	-0.05027
FIL	-0.06482	0.79914*	0.14301
WEB	0.12054	0.39441*	0.13900
TL	0.10265	-0.28263*	0.09968
SV	0.04426	-0.22863*	0.10434
WT	0.03622	-0.17203*	-0.02933
HL	0.04192	-0.16409*	-0.05830
MD	-0.01855	-0.06730*	-0.05556
ID	0.02833	-0.06599*	0.05716
TSN	0.02236	-0.23511	0.59979*
BSN	-0.04093	-0.17333	0.53896*
DSP	0.16315	-0.02634	0.37202*
LL	0.01718	-0.07630	-0.17399*
HW	0.00079	-0.10520	-0.12164*
GI	-0.03347	-0.09673	-0.11640*
AL	0.04642	-0.00895	-0.11168*

Table 2.13 Pooled within-groups correlations between discriminating variables (characters) and canonical discriminant functions. Characters are ordered by size of correlation within function.

T. helveticus. Karyology was carried out on two of these females by Simon Sims (Leicester University), who confirmed their status as *T. vulgaris*. These females generally had no throat spots and few belly spots. The characters which made the greatest contribution to the canonical discriminant functions (Table 2.13) were crest shape (Function 1), filament length (Function 2) and throat spot number (Function 3). The misclassification of female *T. vulgaris* may have been a result of Function 3 identifying throat spot number as an important feature separating the female forms. Overall, this mathematical method produced an accuracy of 98% for classifying aquatic phase animals.

Discussion

Newts in the four groups studied share several morphological traits. All are cryptic when viewed dorsally, but are conspicuous when viewed ventrally. Their dark dorsal colouration probably acts as camouflage to protect them from predators hunting from above during either, or both of their terrestrial and aquatic phases. This is supported by personal observations that newts of these species have demonstrated some ability to vary their dorsal colouration to match that of the environment as closely as possible. Belly colouration, however, does not alter to match the environment and, as newts are known to be noxious, it is possible that their bright ventral colouration has an aposematic function (Beebee 1980).

Aquatic males differ most strikingly from conspecific females in the patterning and colouration of their flanks and tails, which are most conspicuous when viewed dorsally, such as during courtship (Verrell 1982). As female newts, which do not have such conspicuous lateral patterning, do not display, this suggests that male patterning appears to have evolved as a compromise for the conflicting male interests of reproduction and avoiding predation, whilst selection pressures have favoured in female newts a pattern and colouration which has evolved primarily to avoid predation.

In this study attention has largely been focused on distinguishing newts by morpholgical features grouped loosely into body and limb dimensions, integumental character development, spot characteristics and colouration. The work of Veith and Dorr (1985) indicates that other features, including patterning and colouration are worthy of further empirical investigation. These authors report that the rows of pores on the head and the presence of a bright mark near the point of attachment of the hind limbs are

suitable features for the distinction of male and female *T. vulgaris* and *T. helveticus* and they add that the pigmentation of the cloaca is also a useful guide for distinguishing female newts.

The presence or absence of throat spots is one of the more accurate features for distinguishing newts but caution must be exercised when using this criterion as Clifford (1986) found that 26.5% of the female *T. vulgaris* captured in his study lacked throat spots. He noted that all the immaculate-throated females had a yellowish pigment under the skin of the throat which contrasts with the pinky-white throat of female *T. helveticus* which lack this pigment layer. A feature of aquatic female *T. helveticus* which has not generally been cited in classification guides is the presence of a small tail filament. This was noted by Noble (1931) and more recently Veith and Dorr (1985), who reported an incidence of 69.2% in their German populations, with some filaments being over 2mm long. As a large proportion of female *T. helveticus* lack this feature, its absence should not be interpreted as unequivocal evidence of female *T. vulgaris* status. Although there are many morphological features on which newts could discriminate there is no one measure by which humans could reliably discriminate the four forms; a multivariate approach to discrimination is therefore recommended. The discriminant function analysis is a fairly accurate method for classifying aquatic phase newts, however where complete accuracy is required, laboratory techniques such as karyology (e.g. Schmid *et al.* 1979), or electrophoresis (e.g. Rafinski & Arntzen, 1987) are also recommended for extreme morphological variants.

No hybrids were found during this study, which again suggests that natural levels of hybridisation between *T. vulgaris* and *T. helveticus* are low. However, the data set obtained here confirmed intermediate morphology in a

male newt from a pond in mid-Wales, which karyology subsequently proved to be an F1 hybrid resulting from a male *T. helveticus* x female *T. vulgaris* mating (Griffiths *et al.* 1987). In the light of the differing morphology of the subspecies of *T. vulgaris* and *T. helveticus* (Thorn 1968, Raxworthy 1989a), it must be stressed that the data set obtained in this study are only useful as a guide for distinguishing newts thought to be the nominate subspecies or their hybrids, that are captured in areas where they are sympatric.

Newts with well-developed aquatic characters are relatively easy to classify, but exceptions may occur when individuals possess one or more features typical of another form. Roberts and Verrell (1984) found male and female *T. vulgaris* with proximal webbing on one limb. The digits of these atypically webbed limbs were relatively short and after rejecting hybridism or gross chromosome abnormality as possible causes, the authors suggested that these features were a product of abnormal regeneration of a lost limb. Other abnormally short appendages, such as the tail, may also be the result of injury (Harrison *et al.* 1984).

Considerable variation exists in the dimensions of individuals in each of the four groups investigated here which has also been reported by authors measuring other populations (e.g. van Gelder 1973, Harrison *et al.* 1984, Smith 1973, Veith & Dorr 1985). Early reports of the size of *T. vulgaris* and *T. helveticus*, have been summarised by Frazer (1983) and they indicate that considerable size variation exists betweeen indivduals in intraspecific populations. His findings contrast with those of Clifford (1986), Dolmen (1983) and Harrison *et al.* (1984). Although potential discrepancies may arise when the data of authors using non-standard measuring techniques are compared (Harrison *et al.* 1984), these results nevertheless suggest interpopulation variation in morphology may occur.

MORPHOLOGICAL VARIATION BETWEEN POPULATIONS

Where a comparative data set has been compiled from individuals of the same species sampled from different sites at different times, as it has here, it is important to be aware of the extent of interpopulation variation in morphology for several reasons: 1) to determine whether it is necessary to control for differences in the body length of individuals from different populations during data analysis, as many seasonally developed characters may be correlated with size. 2) so that intra-specific population variation may be taken into account when distinguishing different species. Without such information it is difficult to establish whether differences observed between species are a greater reflection of intraspecific population variation, or of true species differences. 3) it provides information to permit valid grouping of intra-specific data from different populations for future analyses where there would otherwise be insufficient data.

Method

To avoid discrepancies resulting from sampling at different times of the aquatic phase, seasonally developed characteristics such as crest height (CH), are best compared between interspecific populations when measured at the same phase (month) of the breeding season; male and female *T. vulgaris* from Littleworth Common (males: n=18, females: n=23) and Oxford (males: n=24, females: n=10) captured in April were the only interspecific population groups of adequate sample size which fulfilled this criterion. Comparisons of SV, WT, GI, TAL, TD, BSN and TSN for both sexes and CH and WEB for males only, were carried out. As differences in snout-vent length (SV) were found between populations, the development of four seasonally developed potential secondary sexual characteristics (TD, CH, WEB, IDL), were corrected for body size (e.g. [WEB x 100/SV]) and tested for

significance between male *T. vulgaris* from Littleworth Common (n=18) and Oxford (n=24).

Results

Although animals from the Oxford population were heavier and of larger size (Table 14 & 15) than those from Littleworth Common, there were no differences in either the absolute (Table 15) or relative (Table 16) degree of male character development, or in the spot paramaters between the two populations.

CHARACTER	SITE	MEDIAN	RANGE	MANN-WHITNEY U TESTS* z	P
Weight	LC	1.70g	1.25-2.84g	-3.84	<0.001
	OX	2.38g	1.51-2.87g		
Girth	LC	22.0mm	18.0-24.0mm	-2.627	<0.01
	OX	23.0mm	20.0-28.0mm		
Snout-vent Length	LC	40.5mm	35.5-47.5mm	-3.503	<0.001
	OX	44.7mm	34.0-50.0mm		
Tail Length	LC	42.0mm	36.5-51.5mm	-3.745	<0.001
	OX	38.5mm	29.5-47.0mm		
Tail Depth	LC	10.25mm	7.5-12.0mm	-0.857	>0.05
	OX	10.58mm	7.5-14.0mm		
Crest Height	LC	1.39mm	0.5-2.5mm	-1.068	>0.05
	OX	1.67mm	0.5-2.5mm		
Webbing Width	LC	0.54mm	0.0-1.50mm	-1.246	>0.05
	OX	0.74mm	0.0-1.25mm		
Belly Spot Number	LC	11	7-22	-1.403	>0.05
	OX	14.5	4-22		
Throat Spot Number	LC	21.5	14-34	-1.821	>0.05
	OX	25.2	15-38		

Table 2.14 Variation in morphology between two aquatic populations of male *T. v. vulgaris* sampled in April. Key to abbreviations: * = two-tailed test; LC = Littleworth Common, Bucks. (N=18); Ox = Oxford, Oxon. (n=24).

CHARACTER	SITE	MEDIAN	RANGE	MANN-WHITNEY U TEST* z	P
Weight	LC	1.55g	1.15-2.77g	-3.313	<0.001
	OX	2.10g	1.69-3.53g		
Girth	LC	23.0mm	19.0-27.0mm	-2.527	<0.02
	OX	25.5mm	21.0-31.0mm		
Snout-vent Length	LC	39.5mm	35.5-48.5mm	-2.846	<0.01
	OX	43.25mm	40.5-48.0mm		
Tail Length	LC	32.87mm	21.5-40.0mm	-2.570	<0.02
	OX	37.25mm	32.5-47.0mm		
Tail Depth	LC	5.79mm	3.5-7.5mm	-2.655	<0.01
	OX	6.92mm	5.0-9.5mm		
Belly Spot Number	LC	18	0-28	-1.863	>0.05
	OX	23.5	7-46		
Throat Spot Number	LC	37	24-73	-1.863	>0.05
	OX	54	0-83		

Table 2.15 Variation in morphology between two aquatic populations of female *T. v. vulgaris* sampled in April. Key to abbreviations: * = two-tailed tests; LC = Littleworth Common, Bucks. (n=23); OX = Oxford, Oxon. (n=10).

Discussion

Various factors may contribute to physical variation between populations, including differences in population age structure, genetic constitution and environmental influences, or a combination of more than one of these. Analysis of adhesion lines between ring bones indicates that only a weak relationship exists between size and age (Halliday & Verrell 1988, Hagstrom 1977 & 1980, Verrell & Francillion 1986) and therefore the differences in size between mature animals from different populations reported here cannot be entirely explained by differences in population age structure.

Genetic constitution may play a major role in interpopulation variation as initial colonisation of a new and relatively isolated pond is most likely to commence with just a few migrant individuals, whose decendants return to the pond of their origin to contribute a high proportion of subsequent breeding individuals in it. The resultant population may have a limited allele frequency, which may differ from other populations and would be unlikely to be affected by fresh variation introduced through later migration (Berry 1977). This phenomenon is known as the 'Founder principle' (Mayr 1954) and may account for some of the phenotypic differences observed between newt populations, such as frequency of throat-spotting (Clifford 1986).

CHARACTER	SITE	RELATIVE DEVELOPMENT Median	Range	MANN-WHITNEY U TEST* z	P
Tail Depth	LC	24.74	19.75-29.87	-1.106	>0.05
	OX	22.93	17.88-31.11		
Crest Height	LC	3.55	1.39-6.10	-0.140	>0.05
	OX	3.58	1.32-5.56		
Webbing Width	LC	1.77	0-3.66	-0.077	>0.05
	OX	1.66	0-2.78		
Intermedium-Digit Length	LC	11.89	11.11-17.72	-1.081	>0.05
	OX	14.29	13.00-17.65		

Table 2.16 Variation in epigamic character development relative to body size between two aquatic populations of male *T. vulgaris* sampled in April. Key to abbreviations: § = calculated from the following formula:- [(Character x 100)/SV]; * = Two-tailed tests; LC = Littleworth Common, Bucks. (n=18); OX = Oxford, Oxon. (n=24).

The expression of some alleles, particularly those relating to growth, may be further modified by environmental influences such as competition and resource availablity. These topics are currently under investigation by J. Baker (Open University) for larval *T. vulgaris*. Overall, a habitat which provides optimum conditions for growth is likely to maximise the growth potential denoted by the genotype. It is therefore plausable to assume that the population of *T. vulgaris* from Oxford either possessed a greater proportion of alleles which code for large size, or had access to a better quality aquatic or terrestrial environment, or a combination of both, than animals of the same species from Littleworth Common.

In this study, the Oxford females which were larger in length were also of wider girth and heavier weight, possibly because they carried more eggs than smaller females from Littleworth Common. This suggestion is supported by Verrell (1986b) who observed a positive correlation between size and the number of yolked oocytes carried by female *T. vulgaris*. The differences found between populations of females in this study are not unique, as Clifford (1986) and Dolmen (1983) noted differences in the weight of females from populations of *T. vulgaris* and *T. cristatus*, respectively.

Certain morphological variations may also be favoured due to positive selection pressure being exerted on individuals with those characters. Chance differences occurring in individuals of the initial population may be magnified through a mechanism of preferential mate choice (sexual selection), until the character becomes fixed in the population. Sexual selection through female mate choice may have led to the evolution of the highly developed secondary sexual characters in male *Triturus* (Halliday 1977a). This topic is discussed further in the following section and it is currently the subject of study by Charlotte Hosie (Open University).

SEASONAL DEVELOPMENT OF CHARACTERS

It is well known that when newts migrate to the pond in the Spring, their aquatic characters are poorly developed. Two reports (Griffiths and Mylotte 1989, Verrell *et al.* 1986) demonstrate that integumental character development in male newts is gradual and takes many weeks before they reak their peak. Integumental character development may be important for reproduction, or an adaptation for life in the water, or may serve a dual function as a combination of both (Beebee 1980, Darwin 1871, Halliday 1972, Halliday & Joly 1981). Features which are proportionately better developed in male newts (e.g. the tail filament in *T. helveticus*), or which are absent in females (e.g. hind limb webbing) probably serve at least in part, as male secondary sexual characters and it has been suggested (Halliday 1972) that they may play a role in reproductive isolation.

The most detailed study of integumental seasonal development to date, that of Griffiths and Mylotte (1989), focuses on male *T. vulgaris* and *T. helveticus*, but does not describe character development in females, or make interspecific comparisons. This section provides an investigation of seasonal development of external characters which may be associated with reproduction in male and female *T. vulgaris* and *T. helveticus* with the aim of gaining a better understanding of their function.

Method

Associations between the snout-vent length (SV) and tail length (TAL), tail depth (TD) and forelimb digit length (IDL) were tested using Spearmans Correlation for terrestrial male and female *T. vulgaris* and female *T. helveticus*. In addition, crest height (CH) and width of webbing (WEB) for males of both species and filament length (FIL) for male and female *T.*

helveticus were also tested. Terrestrial male *T. helveticus* were excluded due to low sample sizes.

As positive correlations were found, the same characters (x100) were standardised for size in each of the four groups. Median monthly relative seasonal development ratios were calculated for each of the aquatic and terrestrial cohorts; the limited terrestrial male *T. helveticus* data have been included for reference. Kruskal-Wallis 1-way Anova (corrected for ties) were carried out (see Siegel & Castellan 1988) using SPSSX between the five cohorts for each of the four newt groups to determine whether significant changes in character development relative to body size had taken place over the months of the aquatic phase. Where significant changes were found, the month of maximum character development is shown.

Results

In male and female *T. vulgaris* and female *T. helveticus* captured on land prior to entering the pond in March, a positive correlation existed between SV and all characters analysed except WEB in male *T. vulgaris* and FIL in female *T. helveticus* (Table 2.17). Hence, larger animals entering the pond at the start of the breeding season generally have better developed integumental characters than smaller animals.

The Kruskal-Wallis tests indicated that there were significant changes in the relative size of most physical characters tested (Table 2.18), with the exception of TAL in male *T. helveticus* and IDL in female *T. helveticus*. Most of the male and female characters studied showed seasonal growth, with the trends particularly strong for TD, CH, WEB and FIL (Figures 2.4 & 2.5). Character development tended to peak in May and was generally followed by a decline in June. Relative homologous character size between

AQUATIC FORM	SPEARMANS CORRELATION	CHARACTER WT	GI	TAL	TD	CH	WEB	IDL	FIL
Male	Coef.	.787	.393	.727	.566	.452	.062	.679	-
T. vulgaris	n	30	30	30	30	30	30	30	
	P	***	*	***	***	*	NS	***	
Female	Coef.	.895	.510	.605	.267	-	-	.263	-
T. vulgaris	n	44	44	44	44			44	
	P	***	***	***	NS			NS	
Female	Coef.	.900	.777	.802	.450	-	-	.420	.145
T. helveticus	n	27	27	27	27			27	27
	P	***	***	***	*			*	NS

Table 2.17 Correlation between character development and body size (SV) in terrestrial newts. Probabilities: NS=>0.05; *=<0.05; **=<0.01; ***=<0.001.

FORM	CHARACTER	KRUSKAL-WALLIS* χ^2	P	MONTH OF MAXIMUM RELATIVE DEVELOPMENT
Male	TAL	12.1826	0.0160	May
T. vulgaris	TD	86.9983	0.0000	May
(n=126)	CH	75.6168	0.0000	May
	WEB	83.5972	0.0000	May
	IDL	10.5751	0.0318	May
Male	TAL	3.8242	0.4303	-
T. helveticus	TD	41.4459	0.0000	May
(n=64)	CH	25.7227	0.0000	May
	WEB	25.8446	0.0000	May
	IDL	8.1413	0.0865	May
	FIL	15.9593	0.0031	May
Female	TAL	11.8420	0.0186	June
T. vulgaris	TD	78.7980	0.0000	May
(n=130)	IDL	13.7658	0.0081	June
Female	TAL	11.9730	0.0176	May
T. helveticus	TD	55.7602	0.0000	May
(n=85)	IDL	7.5252	0.1106	-
	FIL	23.0485	0.0001	June

Table 2.18 A test of changes in character development relative to body size (SV) from March to June in male and female *T. v. vulgaris* and *T. h. helveticus*. Key: * = corrected for ties. These results correspond to the data shown in Figures 2.4 and 2.5.

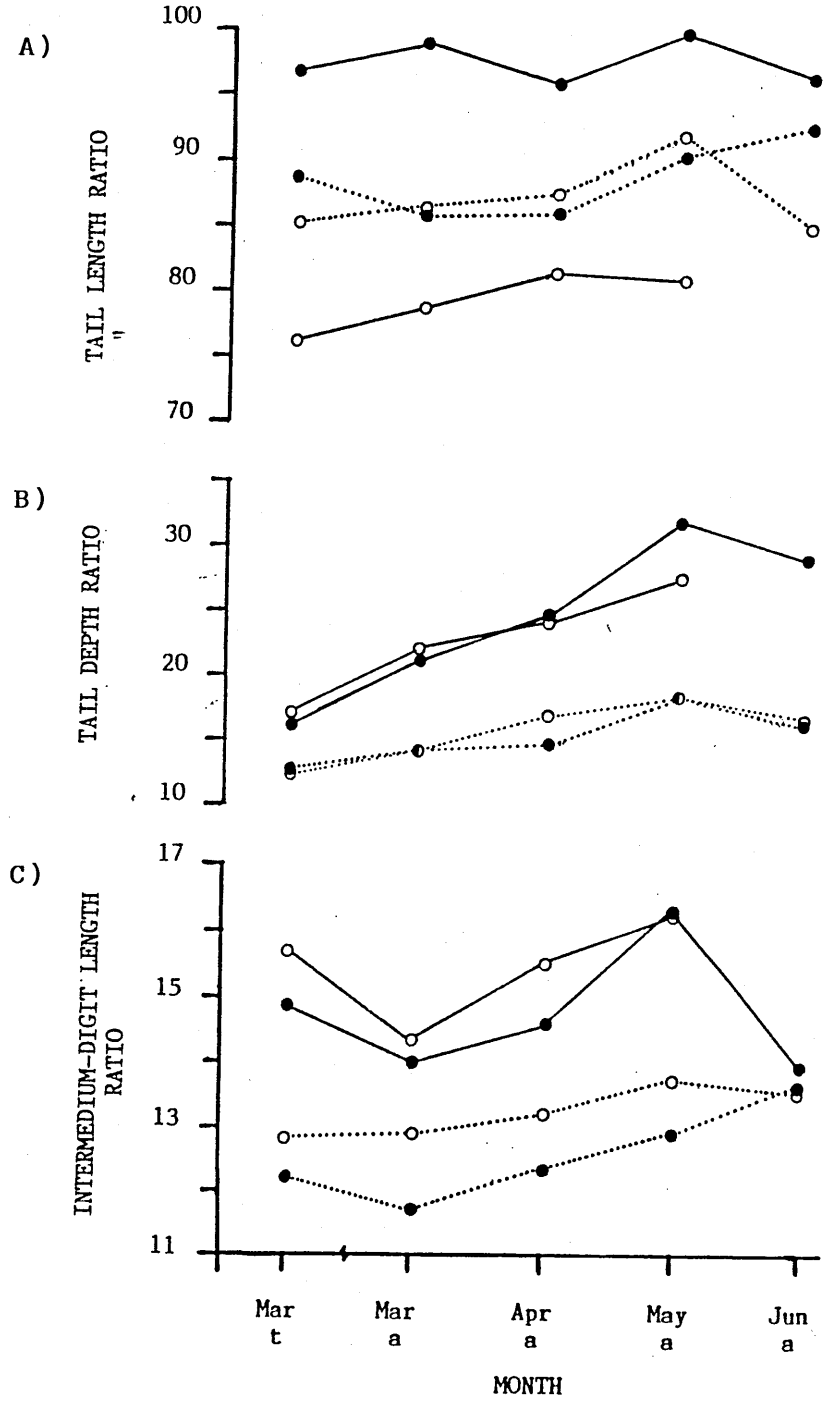


Figure 2.4 Seasonal changes in external morphology relative to body size (SV) in male and female newts (I). Key: A = Tail length (TAL); B = Tail depth (TD); C = Intermedium-digit length (IDL); closed circles = *T. vul*; open circles = *T. helv*; continuous line = males, dotted line = females, t = terrestrial; a = aquatic.

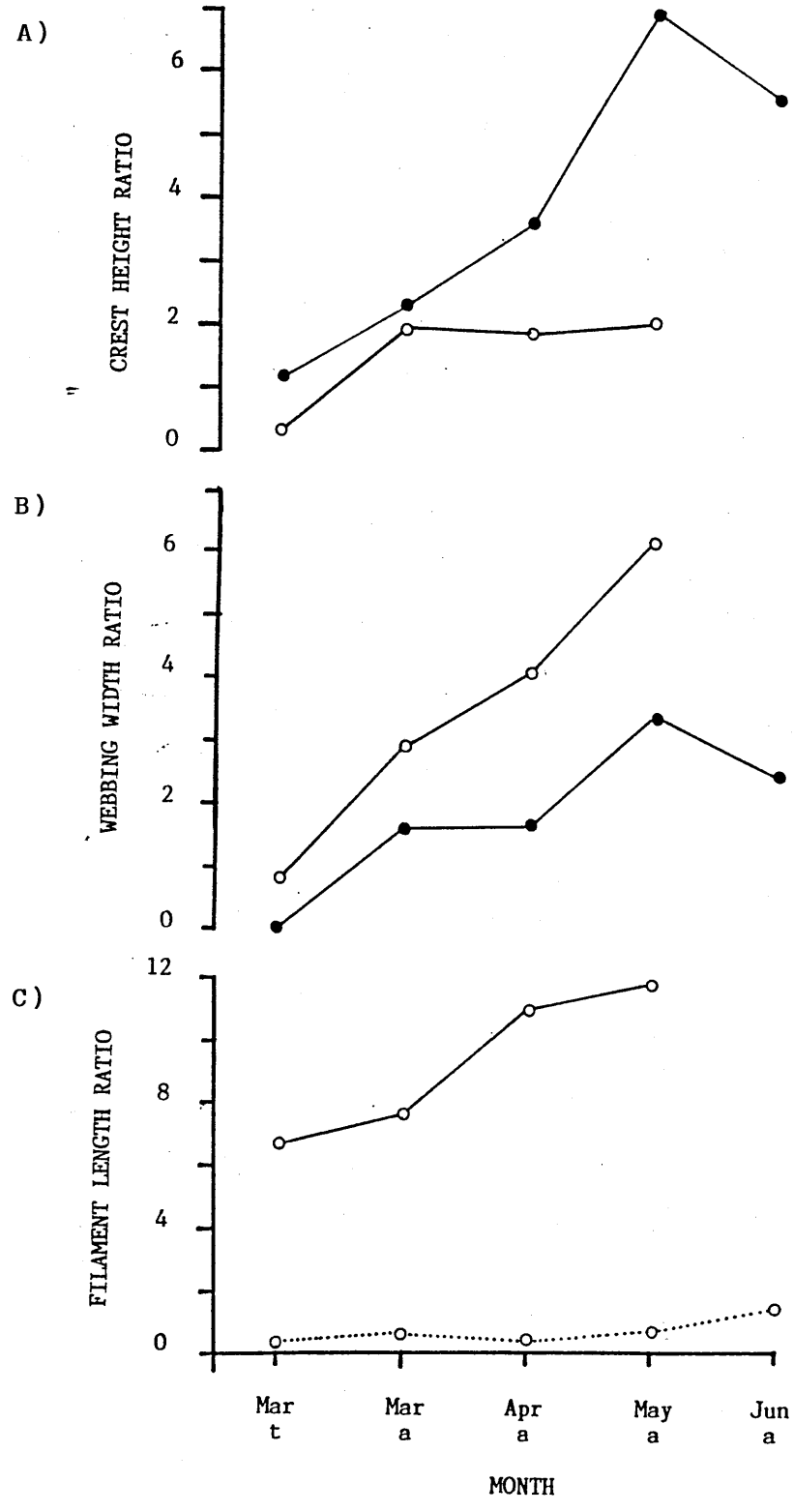


Figure 2.5 Seasonal changes in external morphology relative to body size (SV) in newts (II). Key: A = Crest height (CH); B = Webbing width (WEB); C= Filament length (FIL); closed circles = *T. vul*; open circles = *T. helv*; continuous line = males, dotted line = females, t = terrestrial; a = aquatic.

conspecific males and females was larger in the former sex with the exception of TAL in *T. helveticus*, as males of this species have truncated tails, unlike animals in the other three groups.

Discussion

Griffiths and Mylotte (1989) found that full development of male characters was dependant on their entering the water and postulated that this has consequences for the mating success of males, which usually migrate to the ponds before females (Blab & Blab 1981, Griffiths 1984, Harrison *et al.* 1983, Persson 1946). They suggested that entering the water early enables them to develop their seasonal characters before females become abundant. The results of this investigation clearly show that the development of some integumental characters is not confined to males, but also occurs in females and shows a similar pattern of growth and regression in both sexes. This linkage suggests that characters which are shared by both sexes may also share a common function, at least in part. The development of the tail fin may assist swimming for both males and females, although as it is deeper in male newts, this suggests it may play some role during courtship, possibly by increasing the efficiency of display or pursual of the female.

Other features which are better developed in male newts, or which are absent in females probably also have a reproductive function. This is further supported by reports that courtship activity is at its most intense in May (Verrell & Halliday 1985), which this study confirms is when such sexually dimorphic seasonal features as the male crest and hind limb webbing reach their peak of development. It would be wrong however, to assume that all seasonally developed characteristics function during courtship. The position of the upper mandible membranes around the jaws

indicates that they may be of primary benefit during feeding, possibly acting as a filter to retain prey items in the mouth when water is expelled from the oral cavity during the ingestion process (Joly 1981).

The function of the secondary sexual characters of *Triturus* has been discussed by several authors in recent years. The most important detailed discussions include those of Halliday (1975a), who suggested functional explanations of male secondary sexual characters based on observations made during courtship, Pecio and Rafinski (1985), who made predictions about the courtship display of *T. vulgaris* subspecies based on their aquatic morphology and Raxworthy (1989a), who used a comparative approach between *T. helveticus* and the subspecies of *T. vulgaris* to investigate the relationship between morphology and courtship display.

Halliday (1977a) suggests that the distal tail filament found in male *T. helveticus* may function to reduce water turbulance during courtship, particularly during the fan display. This has also been suggested by Noble (1931) for the races of *Cynops pyrrhogaster* which also have a filament and it is further supported by Raxworthy (1989a) who found a positive correlation between fan duration and tail tip length. Reducing drag on the tail may facilitate courtship in three ways: (i) by producing a smoother, stronger water current which stimulates the female more effectively, (ii) by reducing the effort required to sustain courtship display and (iii) by reducing energy expenditure during swimming, enabling the male to pursue the female more effectively. As females do not normally display to males, the tail filament must convey little direct benefit during courtship to female *T. helevticus*. It may improve the female's ability to swim, both away from unsuitable males attempting to court and in other aspects of daily life, but it is probably a consequence

of genetic correlation between homologous characters in the sexes caused by pleiotropic effects of genes which act similarly in males and females (Lande 1980, 1987).

Potential secondary sexual characters which have not been previously reported include the relatively long forelimb digits of males of both species. They probably enable the male to retain his grip on the substrate during courtship, particularly during the fan display. Observations of males being propelled backwards by the beat of their tail when contact with the substrate is lost support this hypothesis. Harrison *et al.* (1984) also thought that the relative differences in tail length between male *T. vulgaris* and *T. helveticus* are related to courtship, with males of each species possessing the size of tail best suited to their differing courtship acts. Darwin (1871) suggested that hind-limb webbing aids the male in his pursuit of the female, but this is unlikely as the hind-limbs are held against the flanks when swimming, although they may be used to provide initial thrust off the substrate, or stabilisation during courtship (Halliday & Joly 1980, Raxworthy 1989a, Sparreboom 1986), feeding (Halliday & Joly 1980) and decent after breathing (Beebee 1980).

The exact role of secondary sexual characters in courtship has not been investigated although this has been discussed by Halliday (1975a, 1977a) who suggested that these characters may influence female choice of mates. Although there is as yet no published evidence to suggest that female *T. vulgaris* and *T. helveticus* find conspecific males with larger secondary sexual charcters more attractive, Malacarne and Cortassa (1983) observed that male *T. carnifex* with deeper tails had more spermatophores picked up by females than males with less well developed tails. Verrell *et al.* (1986) suggested that the height of a male *T. vulgaris* crest may provide

information to a female about his ability to produce large quantities of sperm as they found that crest height is positively correlated with the proportion of evacuated glandular tissue in the testis. If this ability has a genetic basis then females which mate with males with larger secondary sexual characters may produce offspring which inherit this capacity.

This study indicates that the eventual degree of development of integumental characters is linked to factors other than body size; feeding efficiency may be one of them. This is supported by anecdotal obervations that the secondary sexual characteristics of newts maintained under laboratory conditions regress if an insufficient or unsuitable diet is provided (Halliday 1977a). A newt may possibly be providing a female with information about his ability to aquire resources (feed successfully) by the relative size of his integumental secondary sexual characters, which if genetically based, may convey a tangible selective advantage to the offspring of females preferring males with well developed characters when food is of limited availability, or when storing fat for winter torpor.

For male newts with better developed secondary sexual characteristics to gain a selective advantage through mechanisms of female choice for large characters, two conditions must be fulfilled: (i) female newts must be able to discriminate between the extremes of integumental character development and must exhibit a preference to mate with males with better developed characters and (ii) secondary sexual character development must be an honest advertisement of the heritable qualities carried on the male genotype. There is as yet, no evidence available to suggest that either condition is satisfied in *T. vulgaris* or *T. helveticus*. Indeed, although

female *T. carnifex* picked up more spermatophores produced by males with deeper tails, Malacarne and Cortassa (1983) concluded that this was because males with deeper tails courted female newts more vigorously, which resulted in more efficient stimulation of the female and ultimately increased spermatophore production. Teyssedre and Halliday (1986) observed that male *T. vulgaris* which courted female newts more intensely were more successful in persuading females to mate. These findings suggest that male newts with better developed secondary sexual characters stimulate conspecific females because they court them more vigorously, not because their appearance is intrinsically more attractive to females.

A new question arises, how and when does large character development facilitate courtship? A display phase is commonly ended when the male is forced to return to the surface of the water to take in air. Courtship is a persuit in which great demand is placed on a male's oxygen availablility as it is not only a period of intense activity but it is also a time when the male suppresses the number of breathing ascents he makes (Halliday & Sweatman 1976). In *T. vulgaris*, over 80% of the respiratory capillaries are in the skin (Czopek 1959) and 84% of the total oxygen uptake in the Red Spotted newt (*Notophthalamus viridescens*) occurs via cutaneous exchange (Ultsch 1976): cutaneous gas exchange is clearly important in aquatic newts.

By developing vascularised integumental characters, a male may increase the area of his body over which oxygen is absorbed (Arnold & Burton 1978, Foxon 1964, Halliday 1977b) thus increasing his surface to volume ratio. Hence a courting male with well developed crest and webbing would absorb more oxygen from the environment than a male of similar size with poorly developed characters, effectively supplementing the reserve of oxygen held

in the lungs a time when demand is at its greatest and supply is at its most critical. This may in turn enable a male with large characters to court a female more vigorously or for longer than one with poorly developed characters. Indirect evidence to support the hypothesis that integumental characters increase the ability of male newts to absorb oxygen from the water has been provided by R. Griffiths (pers. comm.), who noted that female newts are more likely to be casualties of submerged traps without air pockets than males, despite the latter being the more active sex (Griffiths 1985).

DIFFERENCES IN MORPHOLOGY - AN OVERVIEW.

The evidence presented in this chapter indicates that insufficent distinguishing characters are present in larval forms to enable feasible searches for juvenile hybrids to take place if based on morphology alone. However, the lack of morphologically intermediate adult forms found is in agreement with observations by other authors that hybridisation between these two species is rare (Chapter 1).

Many morphological differences exist between male and female *T. vulgaris* and *T. helveticus* by which males and females of both species may visually discriminate. A multivariate approach to discrimination is more reliable for humans and it therefore may also be more reliably used by newts than a discrimination based on one or two characters, although this latter possiblity cannot be discounted without further investigation. The secondary sexual characters of males of both species differ in form and may therefore provide information for female discrimination between males of different species. However, they may also play a role in female choice between conspecific males, or function more in inter-male competition

during courtship. It is unlikely that these potential functions of morphological differences form mutually exclusive processes, rather that some or all of them interact concurrently; the exact nature of the interactions provided by differing morphological characters during courtship awaits further study.

CHAPTER 3

THE COURTSHIP BEHAVIOUR OF SMOOTH AND PALMATE NEWTS

Early accounts of the sexual behaviour of *T. vulgaris* and *T. helveticus* have been reviewed by Halliday (1974) who concluded that courtship involves a period of prolonged male display which delivers visual, olfactory and tactile stimuli to the female. The courtship displays of the two species are similar as males of both species employ distinctive tail movements known as the wave, the whip and the fan (Halliday 1972, 1974), but quantitative and qualitative differences exist between the homologous display acts of these two species (Halliday 1977a). In recent years a fourth display act called the undulation distale by Wambreuse and Bels (1984) and the distal lure by Sparreboom and Arntzen (1987), now known as the wiggle (agreed at *Triturus* Conference, Leicester, 1984), has also been recognised in *T. vulgaris* and *T. helveticus*.

Differences in courtship behaviour may act to reduce or prevent interspecific hybridisation and it is an ethological mechanism of this nature which is thought to be the primary mechanism responsible for reproductive isolation between *T. vulgaris* and *T. helveticus* (Halliday 1977a, Wambreuse & Bels 1984). Before meaningful analysis of the differences in courtship behaviour between *T. vulgaris* and *T. helveticus* can be made, it is first necessary to have a thorough understanding of the courtship displays of the two species in question. At the time of this study, many aspects of the courtship behaviour of *T. vulgaris* in the laboratory (e.g. Halliday 1972, 1974, 1975b, 1977b; Verrell 1983, 1984, 1986a, 1986b) and the field (Verrell & McCabe 1988, Griffiths 1985) had been well documented, but relatively little empirical investigation of *T. helveticus* courtship behaviour had taken place. The most detailed recent

account and causal analysis of *T. helveticus* courtship has been provided by Wambreuse and Bels (1984).

The aims of this study are fourfold. First, to provide a quantitative and qualitative account of the behaviour of *T. helveticus*. Secondly, to investigate the most recently recognised courtship act, the wiggle, in more detail. Thirdly, to gain an indication of the variation that occurs during courtship and fourthly, to compare aspects of the courtship behaviour of *T. vulgaris* and *T. helveticus* which may play a role as an ethological isolating mechanism. Models of sexual isolation are proposed.

THE COURTSHIP BEHAVIOUR OF *T. H. HELVETICUS.*

This section provides a generalised account of *T. helveticus* courtship, an analysis of male and female behaviour performed in association with the wiggle and an investigation into the variation found in *T. helveticus* encounters.

General methods: Collection, maintenance and recording of animals.

Aquatic phase *T. helveticus* were collected by dip-netting and underwater trapping from the ponds listed in Table 2.5 and also from the Isle of Rhum (Scotland) and the sexes were maintained in the laboratory at 10°C±2°C in separate aquaria containing waterweeds and either aged tap-water or pondwater. Illumination was provided by 'Trulite' fluorescent tubes which emit the same mix of wavelengths found in natural sunlight and the animals were kept on a light regimen that loosely followed the natural photoperiod. They were fed daily on an *ad libitum* diet that consisted predominantly of earthworms, *Tubifex, Chironomid* larvae and *Daphnia* as available.

Pairs of animals with no obvious physical defects (e.g. abnormal eyes) were placed in an aquarium at 18-22°C where they were allowed to move about unrestricted. Recordings of their courtship behaviour were made during the breeding seasons (March to June) of 1982-84 using a Sony time-lapse video recorder with simultaneous spoken commentary. These audio-visual accounts were later transcribed onto paper with the timing of behavioural acts noted to the nearest 0.1 second. Unless otherwise stated, these methods form the basis by which animals of both species used in future experiments were collected, maintained and recorded.

A typical T. helveticus courtship encounter.

Method

Based on observation of 31 courtship encounters involving 28 different pairs of *T. helveticus* using no more than two courtship encounters for any one pair, the series of acts performed during courtship is described in the generalised context of a stylised encounter. The different stages of courtship were distinguished as follows:-

An encounter. An encounter began when two partners met and one or more positive acts were performed by at least one of them toward the other within a distance of 10cm. A courtship encounter included at least one of the most easily recognised courtship acts, whip, fan, or creep. An encounter ended when one of the partners moved away from the other and interaction between the two had not re-commenced within two minutes.

A sequence. The series of acts between successive spermatophore transfer periods was defined as the courtship sequence. Not all courtship attempts by the male resulted in spermatophore deposition. Those that did have been termed 'complete sequences' and those that did not 'incomplete

sequences'. The latter finished with the last act performed by one of the partners at the end of an encounter. They may or may not have been preceded by a complete sequence, which was said to have ended after the female's response to the last push-back (and wiggle) by the male after a spermatophore had been deposited.

A phase. Halliday (1974) noted four phases during a courtship sequence, each of which may be loosely defined by the behavioural acts performed, usually by the male. The phases are: (i) orientation, when the male attempts to take up his position in front of the female, (ii) static display, when the male courts a stationary female, (iii) retreat display, when the male moves backwards whilst displaying before an approaching female and (iv) spermatophore transfer, when a spermatophore is deposited and may be transferred to the female.

Departures in terminology used here from those used recently by Halliday (1974) and Wambreuse and Bels (1984) for homologous *T. vulgaris* and *T. helveticus* behaviour are described.

Results

A T. helveticus courtship encounter.

A courtship encounter is most commonly initiated by a male newt after a chance meeting with a female as they wander around the aquarium but on occasions the male may advance directly towards a moving female, adopting a stance with raised head and trunk (Alert Posture - AP) before moving towards his partner in short leaps from some distance away. When the male is within a few centimetres of the female he often stretches out his head whilst undulating his throat (Sniff - Sn) so that his snout is near to, or touching part of her body. After this initial sniffing bout the

male will manoeuvre himself into a suitable position for courtship by swimming in an arc to face the female in an action known as move-to-the-front (M); and if the female remains stationary the male will continue to display with a series of display acts. However, the female is not always initially responsive to the male and while he is taking up his position in front of his partner, or shortly after he begins to display, an unreceptive female will orientate her head away (Negative Head Turn - Oh-), swivel in the opposite direction (Sw-), or will walk or swim away (Go). If she has not moved too rapidly for him to follow, he will pursue her (Follow - Fo) and enter into another phase of orientation with bouts of sniffing which may continue for several minutes until the female remains stationary once more.

A static display phase then follows with the male courting the female continually, performing a series of tail movements whilst retaining the same position on the substrate. The most easily recognised of these actions is fanning (F) and during this display the male's fore and hind limbs are outstretched, with the digits of the former grasping the substrate whilst the last 1cm of tail are moved rapidly in and out of the depression below the dorso-lateral fold formed by the curvature of the body. As indicated by moving particles of detritus in the water, the rapid oscillation of the tail moving over a total distance of about 1cm per beat, creates a water current, which, because of the stance adopted by the male, is directed from the region of his pelvic girdle, along the channel formed below the dorso-lateral fold towards the female's snout.

After a period lasting several seconds the male will cease fanning and perform one of the three other display acts: the wiggle (Wi), the whip (Wh), or the wave (Wa). During the wiggle the male upturns the distal

portion of his tail and undulates the tip from side to side, usually whilst still in the fanning position. This relatively subtle act is described in greater detail in the following section. It contrasts markedly with the whip which involves a violent lash of the entire tail. Although the male does not usually touch the female during this latter display, the action is frequently so powerful that the female is knocked backwards a few millimetres by the force of the water current produced. The whip often appears temporarily to impede the females progress away from her partner, particularly when it follows in sequence after a move-to-the-front. Any single horizontal swaying movement of the distal part of the tail performed by the male that does not conform to the criteria for any of the other acts has been classed as a wave. The most conspicuous type of wave involves lateral displacement of the male's pelvic girdle away from the female and a simultaneous, large amplitude sweeping movement of the entire tail towards her. [Note: Raxworthy (1989a) has made a distinction between waves and a 'right angle tail hold' in *T. v. vulgaris* display. During the latter act the tail is held quite still and straight at about 90° to the body. He reports that this act does not occur in *T. h. helveticus* courtship.]

After a period of static display incorporating some or all of these acts, a responsive female will turn her head and/or body towards the male and after further courtship will approach him directly (Positive Advance - +Adv). The male will continue to display, moving backwards as he does so (Retreat - R). This movement may occur with no other action, or as the male fans (Retreat & Fan - RF), whips (Retreat & Whip - RWh) and waves (Retreat & Wave - RWa). During retreat and fan and retreat and whip the male loses the contact between his forelimbs and the substrate and is propelled backwards by the beat of his tail. This contrasts with retreat

and retreat and wave behaviour, where the male steps backwards and contact with the substrate is not lost. Such behaviour marks the start of the first retreat display phase but, if the female becomes unresponsive again, the male may revert at any time to orientation or static display behaviour. Sustained approach by the female during the retreat display phase will usually result in the male turning through 180° to begin the creep (C): the start of a series of largely sterotyped acts comprising the spermatophore transfer phase.

During the creep the male squats low to the ground and swings his pelvic girdle from side to side whilst taking short steps forward over a distance of between 5 and 15cms. He will then stop advancing and begin to quiver (Q) his tail from side to side whilst keeping his body motionless and held low. At any time during courtship, but most commonly around the early stages of the spermatophore transfer phase he may release a bubble of air form his mouth (Guff). The female will continue to walk toward the male before brushing her snout against his quivering tail tip (Tail Touch - TT). He responds to this by drawing in his entire tail (folding it like a concertina) which she touches with her snout. This tail touch is the signal for the male to raise his folded tail (Halliday 1976) and deposit a spermatophore (S↓).

Once spermatophore deposition has taken place, the male then walks on in the same shuffling gate as the creep (Creep-On - CO) for about one body length whilst turning his body through 90° into the braking position (Brake - Br), where his tail is moved forward and held folded parallel to the body on the side facing the female. He maintains a stationary body except for the last 1.5cm of his tail which he upturns slightly (about 45°) and undulates slowly over a distance of 1cm, sometimes wiggling his

tail tip and filament at the same time. The female walks towards the undulating tail tip held perpendicular to her by the braking male and, as she does so, her cloaca moves over the approximate area where the spermatophore was deposited. If the spermatophore brushes against her cloaca it will usually adhere, but at no time does the female give any detectable signal relating to the success of this process, known as spermatophore pick-up (S↑). The female then pushes her snout against the male's tail (TT) until he flexes it against her snout with sufficient force to push her backwards (Push Back - Pb) about 1cm. Push-backs may occur several times or not at all. After a tail touch and push-back exchange has ended, the male will usually re-orientate towards the female and begin to display once more, utilising a similar repertoire of display acts as in the earlier sequence, until several spermatophores, each interspersed by display bouts, have been deposited.

Once a number of spermatophores have been deposited the female will become unresponsive again and will subsequently move around the tank apparently ignoring her partner whilst scraping her cloaca on the substrate to remove the remains of any spermatophores picked up earlier. He will usually follow her (Fo) displaying infrequently, but often engaging in prolonged sniffing bouts which appear to be directed particularly towards her cloaca and the areas of her trunk, belly and tail near her cloaca. Eventually he will either ascend for air and fail to find her on his descent, or he will move away (Go) and not return, or he will no longer follow her. This usually marks the end of the sexual encounter.

Discussion

The contrast between the complex series of orientation and courtship display acts performed by males and the relatively limited number of

predominantly orientation acts which compose the majority of female behaviour during a *T. helveticus* courtship encounter, indicates that in common with many species, it is the male which plays the more active role during courtship. The functions of courtship display (Tinbergen 1953, Trivers 1972 - ref. Chapter 1) in newts have been discussed by Halliday (1977a). He concluded that orientation (in the sense of mate location) was not an important aspect of courtship and that in the absence of extensive parental care, the assessment aspect could be confined to assessing the potential genetic fitness of a mate. Causal relationships between the actions of partners during *T. vulgaris* courtship (Halliday 1975b) indicate the synchronisation function, but Halliday suggested that persuasion is the primary function of the male's display.

The persuasive function of *T. helveticus* courtship display is shown here by the change in female behaviour from unreceptive to receptive. The varying male courtship acts provide the female with different sensory information which, probably in combination, act initially to supress the female's tendency to move away from the male and subsequently to stimulate her to approach her partner (Halliday 1977a).

The wiggle, in common with other display acts, probably conveys visual stimulation via movement. During the wave and the whip, the distal portion of the tail is held away from the body and, therefore, may also provide stimulation via colour, contrast and patterning. Both the whip and the fan produce a water current which is directed towards the female and may further carry olfactory signals and provide tactile stimulation of the female's lateral line organs.

The stance of the male during courtship may provide the female with additional visual stimulation relating to integumental character development, colour, form and patterning, which may also be utilised by females to assess the quality of a potential mate, although there is currently no published empirical evidence to suggest that this is the case. By engaging in a prolonged encounter in which several spermatophores are deposited, the female may obtain sperm from males which are amongst the most reproductively prolific and if this tendency has a genetic basis, she may ultimately convey this advantage to her offspring (Halliday 1983).

The movement of some males toward a female from some distance away at the start of an encounter and the occurrence of sniffing behaviour suggest that both visual and olfactory cues are important to male *T. helveticus* in the early stages of an encounter. Visual information probably aids the male to orientate towards the female's head and so to take up the most suitable display position, whilst olfactory cues may provide a variety of information relating to the female's species and reproductive condition. The response of the male to movements of the female during display phases indicates that visual cues continue to be important, whilst the performance of the tail touch after the quiver and brake indicates that tactile cues are also utilised by males during the spermatophore transfer phase.

The primary function of courtship is the transfer of sperm and the subsequent fertilization of ova (Tinbergen 1953). As applied to a male newt, his behaviour suggests that his goal in practice is to fertilize the female's eggs by achieving the successful transfer of at least one spermatophore before his oxygen supply is exhausted. However, as a consequence of the restricted supply of both gametes and spermatophores (Halliday 1976, Verrell 1986a, Verrell *et al.* 1986) there is probably

strong selection pressure on males to ensure that the transfer of spermatophores is as efficient as possible (Halliday, in press). This is probably the primary function of most of the male acts carried out during the spermatophore transfer phase.

It is a characteristic of many species that the sex which has the most limited reproductive potential is also the most selective in their choice of mates (Trivers 1972). As the sperm contained within a spermatophore is thought adequate to fertilize a female's entire compliment of yolked ova (Smith 1973) and as males may deposit spermatophores in many encounters during a breeding season, this suggests that males have the higher reproductive potential, despite the constraints placed upon them by their limited spermatophore production. Hence, it is not surprising that it is males which invest considerable time and energy in courting females whereas females, which make the higher reproductive investment, are slow to become receptive to male courtship and frequently do not become receptive at all.

Departures in terminology.

Halliday's (1972, 1974) terms for *T. vulgaris*, on which Wambreuse and Bels' appear to be based, apply equally well to homologous *T. helveticus* behaviour and therefore I have maintained his terminology wherever possible. Departure from his terminology (Table 3.1) includes the introduction of the wiggle, the distinction of retreat and fan (RF - where a male may move backwards during all or part of a fan bout), retreat and wave (RWa - where a male steps backwards whilst waving) and, due to a desire to describe acts in greater detail to facilitate future analysis, the expansion of the categories of orientation behaviour. Here, departures include the addition of the terms 'head turn' (Oh), 'swivel' (Sw) and

'orientate' (Or). The former two of these actions may be either positive (Oh+, Sw+), or negative (Oh-, Sw-), dependant on movement toward, or away from a partner, respectively.

Behavioural Act	Abbreviation	Halliday (1974)	Wambreuse & Bels (1984)
Advance	Adv	-	-
Alert Posture	AP	+	+
Head Turn	Oh (+/-)	-	-
Positive Advance	+Adv	-	-
Swivel	Sw (+/-)	-	-
Orientate	Or	-	-
Follow	Fo	+	-
Move-to-the-front	M	+	+
Go away	Go	+	-
Wave	Wa	+	+
Whip	Wh	+	+
Fan	F	+	+
Wiggle*	Wi	-	+
Turnabout	@	+	+
Retreat	R	+	+
Retreat & Wave	RWa	-	-
Retreat & Whip	RWh	+	+
Retreat & Fan	RF	-	+
Vacillate	V	+	-
Creep	C	+	+
Quiver	Q	+	+
Guff	Gu	+	-
Touch Tail	TT	+	+
Spermatophore Deposition	S↓	+	+
Creep-On	CO	+	+
Braking	Br	+	+
Spermatophore Pick-up	S↑	+	+
Push-back	Pb	+	+

Table 3.1 Comparison of behavioural terms. As homologous acts are found in both *T. vulgaris* and *T. helveticus*, no distinction at the species level is necessary. * = called 'undulation distale' by Wambreuse and Bels.

Also, I have made a distinction between the positive act of a female approaching a male directly (+Adv) and one where the female advances forward, but the action is not directed at her partner (Adv). The latter may occur for example, when the female cuts across the line of movement of the male. Evidence to support the need for this distinction is provided by analysis of male behaviour following female orientation acts. Figure

3.1 compares the probability with which the male's first action (grouped into the categories of negative orientation, positive orientation, static display, retreat display, spermatophore transfer behaviour and sniffing) occurs after a female performs advance behaviour, with those after she performs directed movement toward (+Adv) and away (Go) from her partner. When the female moves away from the male (Go), his first succeeding behaviour is most commonly to perform an action with a positive orientation component (e.g. Sw+, M) and when the female moves directly towards the male (+Adv) his most common succeeding action is to perform a retreat display action (e.g. RWh). However, when the female simply moves forward (Adv), the male is equally likely to perform either of these two categories of behaviour. This suggests that the male sometimes subsequently acts as if it were a positive action and sometimes as if it were a negative action. Thus for the purposes of analysis, advance cannot be categorised *a priori* as either positive or negative female behaviour.

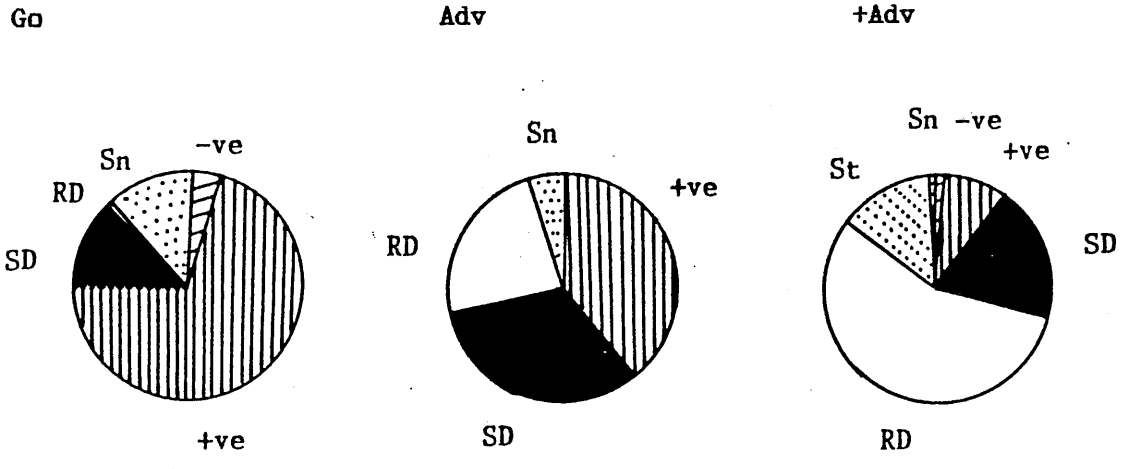


Figure 3.1 Male behaviour after a female advance (Adv) compared with that after female movement directly toward (+Adv) and away (Go) from the male. Segments of pie charts represent the proportion of male behaviours performed. Where: SD = static display, Sn = sniffing, St = spermatophore transfer acts, RD = retreat display, -ve = negative orientation behaviour, +ve = positive orientation behaviour.

THE WIGGLE

The behaviour of the partners during courtship suggests that wiggles serve to re-attract a female that is becoming unresponsive to a male. Preliminary analysis of female behaviour by Wambreuse and Bels (1984) shows that the female most commonly performs positive behaviour in response to a wiggle. However, a detailed investigation of the causal relationship between male and female acts in association with the wiggle is currently lacking, although such analysis has been carried out by Halliday (1975b) for other important display acts. The aims of this section are twofold. First, to provide a qualitative and quantitative description of the wiggle and secondly, to carry out a test of the hypothesis that wiggles are performed in response to changes in female behaviour and that changes in the female's movements are brought about in response to this male action. This is achieved by examining the sequential and temporal transitions between the partners' behaviour. Possible functions of the wiggle are proposed.

Method

The wiggle is qualitatively described in the context in which it is performed during a courtship encounter. Using the data from the eight timed encounters recorded earlier and a further 30 untimed courtship encounters (which were recorded as a spoken commentary in order to gain more information about wiggles and as part of a separate experiment), quantitative details were established which included the proportion of encounters in which wiggles were performed and the sequential and temporal patterning of male and female behaviour performed both in association with the wiggles and during equivalent stages in an encounter where wiggles were not performed.

A description of the wiggle.

The wiggle is a subtle male display act performed with outstretched limbs, whilst static and in a position either facing (after fanning) or perpendicular (after braking) to the female. Once the tail is held parallel to the flank on the side nearest to his partner, the male curls the terminal portion of his tail upwards so that the filament and last few millimeters of tail are held vertically, whilst the bulk of the tail is held horizontally (Figure 3.2). The end of the tail is then undulated repeatedly for several seconds and this action sweeps the tail filament from side to side giving the distal portion of the tail the appearance of a small, wriggling worm. When the male has ceased to undulate his tail tip, it is returned to its horizontal position. The distal portion of the tail may then be swung out 90° in a 'showing-off' motion. During the wiggle the female may perform a variety of orientation acts ranging from lunging toward the tail tip to slowly turning her head away.

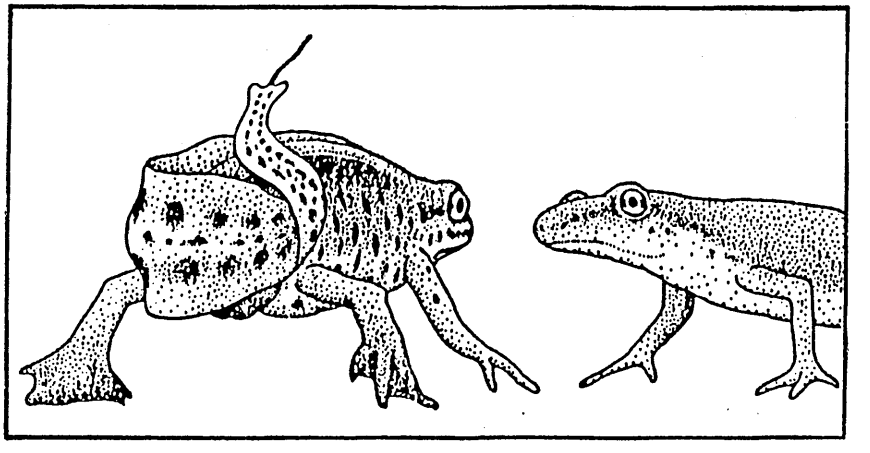


Figure 3.2 Male stance during the wiggle following a fan bout.
The male is on the left. *Drawing by T.R. Halliday.*

Quantitative detail and causal analysis.

The wiggle was performed in 84.2% of the 38 courtship encounters (median per encounter = 5.5, range 0-18) exclusively by males. That the wiggle is an independent action and is not invariably associated with one act is confimed by the results of Table 3.2, which shows that it follows a number of male movements. However, it is clear that a strong relationship exists between wiggles and the prior performance of fanning display. Despite the high probability that a fan bout will precede a wiggle (92.6%), there is a much lower probability that a wiggle will be performed after a fan bout (10.9%, n=1836 F & RF bouts). This suggests that additional factors to those internal to the male may influence the behaviour he performs after a fan bout has ended and Halliday's (1975b) findings indicate that it is female behaviour which may play such a role.

	Male Action			
	F/RF	Br	Wa	Sw+
% Frequency	92.6	6.5	0.5	0.5

Table 3.2 Male action preceding the wiggle. (n=216 wiggles).

Analysis of male behaviour.

In between any two consecutive male acts, a female act may be performed, for example, between the start and end of a fan bout. The transition between male acts may be classed as being uninterrupted, positively interrupted, or negatively interrupted, dependant on female stasis, movement toward, or movement away from the male, respectively. Once the male has commenced fanning, it is inevitable that his next act must be to stop fanning. Thus between the onset of a fan and the performance of his next display act there are two transitions between which the three female

interrupt conditions may apply and must be considered. These are during the fan bout but before the male stops fanning and between the end of the fan bout and the next male action. Halliday (1975b) found that a male *T. helveticus* is very likely to stop fanning if the female shows any behaviour during the fan bout and his data strongly indicate that the female's behaviour influences the temporal patterning of the male's fanning behaviour, but does the behaviour of the female during the fan bout also influence which subsequent behaviour a male performs after a fan bout has ended?

At this point, two conditions applicable to the temporal spacing between the end of a fan bout and the next male action are worthy of consideration:-

1) That the interval between the end of a fanning bout and the next male act is too short for a female to respond. Thus, the outcome of the transition would not be dependant on what the female does immediately after a fan bout, but may be consequent on the male's first act (to stop fanning), possibly in combination with earlier female behaviour. This condition would be characterised by a short transition interval.

2) That the temporal spacing between the end of the fan bout and the ensuing male act is sufficient for the female to act, but that the transition outcome is either (a) dependant on the first male act in combination with earlier female behaviour and is characterised by a long interval due to internal programming of the male, or, (b) is in response to the female's actions subsequent to the fan bout ending.

Almost all wiggles are performed in the absence of female interruption after the fan bout has ended (98.2%), which suggests that condition 2(b) is not the primary operative mechanism which influences the onset of

wiggle display. To determine which of the other two conditions is satisfied for wiggles, it is necessary to compare the interval from the end of the fan bout to the probability of ensuing uninterrupted male acts occuring under the three categories of female interruption during the preceding fan bout. In this analysis, data associated with those fan bouts during which advance (Adv) behaviour was performed by the female were ommitted due to the author's inability to assign this act *a priori* with either positive or negative interrupt status. Only those categories of male behaviour with an overall probability in excess of 10% of the total are presented.

The results of Figure 3.3 show that the probability of each of these transitions follows a different temporal distribution and that their relative sequential importance varies with the preceding fan bout interrupt condition. Immediately after a fan bout during which the female remained static, there is a high probability that a wiggle will be performed. This contrasts with the same period after positively and negatively interrupted fan bouts, where there is a reduced incidence of wiggling and a high probability that a male will perform RWh and positive orientation acts, respectively. As these behaviours decline with lengthening interval duration, there is an increasing probability that other male actions will occur. The temporal transitions to wiggles, RWh's and some early orientation acts appear to be timed to allow the female sufficient time to act and so satisfy condition 1. These data therefore suggest that the variation in the probability of individual male acts suceeding fan bouts is partly explained by female behaviour during the fan bout, where some acts, such as wiggles, RWh and some orientation acts are performed without further assessment of the female's behaviour, and partly by factors internal to the male, where further assessment is possible after a longer interval has elapsed.

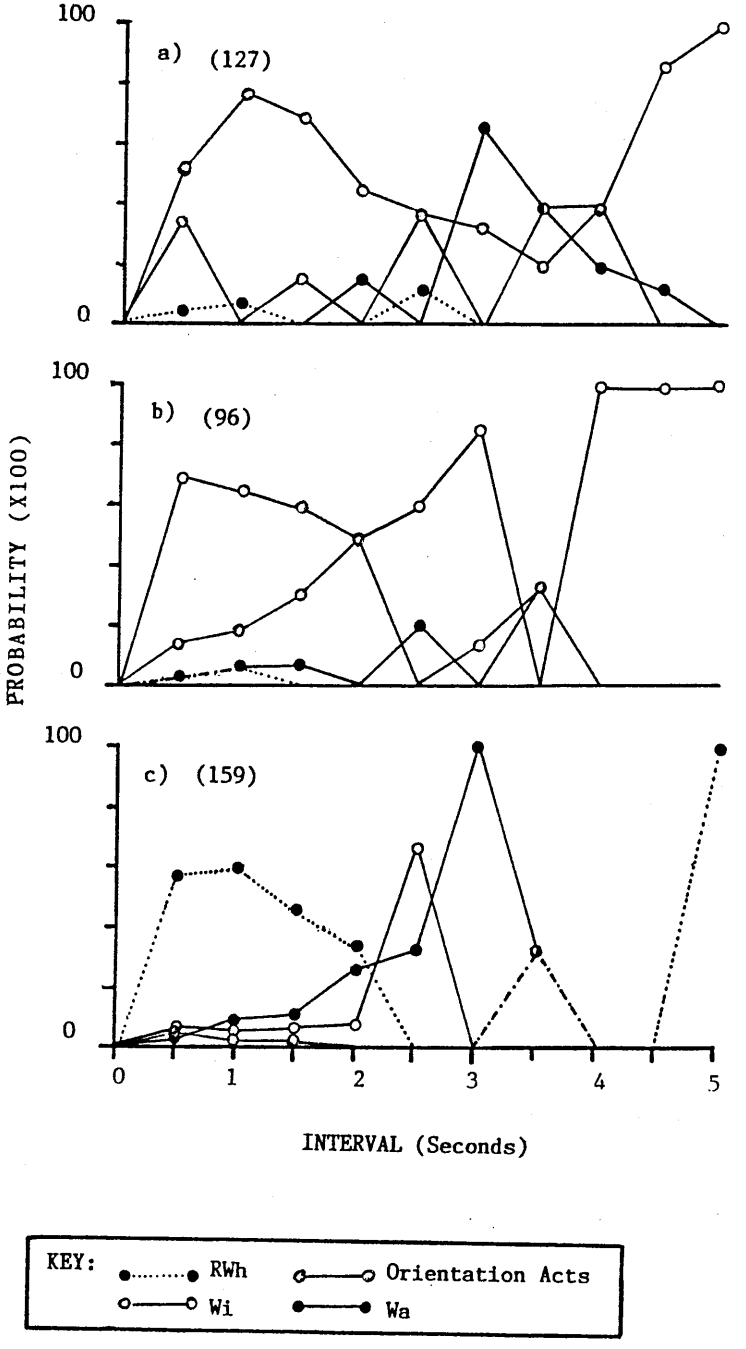


Figure 3.3 Probability distributions of transitions following the end of a fan bout. a) Negatively interrupted transitions; b) Uninterrupted transitions; c) Positively interrupted transitions. Bracketed figures are the numbers of observations on which the plots are based.

The data indicate that the nature of female behaviour performed during a fan bout plays a role in increasing the probability of whether a wiggle will be subsequently performed. Unresponsive female behaviour appears to be a particularly important influence, as there is no female movement during 57.5% of the fan bouts which precede a wiggle, but the incidence of wiggling after negative interruptions suggests that some unreceptive female movements may also play such a role. Analysis of the frequency of a male behaviour occurring after a fan bout in which a particular category of female behaviour was performed (Table 3.3) reveals that the wiggle is the most common transition (33.6%) after the female interrupts a fan with a negative head turn (Oh-), which is the weakest unequivocal negative movement a female may perform. Hence, these data in combination suggest that the male performs a wiggle in response to unresponsive, or weakly unreceptive female behaviour during a fan bout. It must be stressed that the wiggle may not be purely a consequence of female behaviour performed solely during the fan bout and that it cannot be discounted that this transition in male behaviour may also be due in part to an earlier female act, or to the cumulative effect of a combination of earlier female acts.

If one of the functions of the wiggle is to elicit a response from the female, it would be predicted that there would be an adequate latency from the wiggle to the ensuing male action to enable female movement to take place. Before analysis of the influence of wiggling on female behaviour, it is necessary to investigate the temporal spacing between the first and second male action after a fan bout has ended, which, for the purposes of analysis, defines the interval available for subsequent female movement. As the transition to the wiggle is usually uninterrupted, a comparison of the cumulative probability of the interval from the uninterrupted first male action to his uninterrupted second action has been made for male acts

with a frequency of 17 or more (Figure 3.4). These data clearly indicate that there is a relatively long and often substantial transition interval following the wiggle. This suggests that the male is internally programmed to allow the female more time to respond after the wiggle than after most of the other actions he may perform following the end of a fan bout.

	FEMALE BEHAVIOUR DURING FAN BOUT											
SUCEEDING MALE ACTION	Go	Sw-	Oh-	Sta	Adv	Oh+	Sw+	Or	+Adv	Sn	Other	Total
Go	4	1	1	6	1	1	0	0	8	0	0	22
Sta	25	17	34	118	14	27	1	0	85	0	1	322
Oh+	3	1	0	0	0	0	0	0	1	0	0	5
Sw+	13	2	1	2	2	0	0	0	1	0	0	21
@	1	0	0	47	3	4	2	0	25	0	1	83
Or	22	6	8	13	5	3	0	0	0	0	1	58
+Adv	9	4	9	41	1	0	0	0	1	0	0	65
Fo	30	3	1	0	0	0	0	0	0	0	0	34
M	45	14	11	6	22	1	0	0	1	0	2	102
Wa	3	1	12	143	9	12	0	0	21	1	3	205
Wh	8	2	7	47	15	21	0	2	49	0	1	152
F	0	0	3	25	2	1	1	1	9	1	0	43
Wi	2	8	47	115	6	6	3	0	8	0	5	200
R	0	0	1	1	3	1	1	0	19	0	0	26
RWh	0	1	5	19	22	33	5	0	319	0	0	404
C or V	0	0	0	6	0	0	1	0	68	0	0	75
Sn	2	0	0	3	2	0	0	0	6	0	2	15
Other	0	0	0	0	0	0	0	0	4	0	0	4
Total	167	60	140	592	107	110	14	3	625	2	16	1836

Table 3.3 Male behaviour after the end of a fan bout. For key to abbreviations see Table 3.1 except for Sta (stasis) = female remained static during the fan bout.

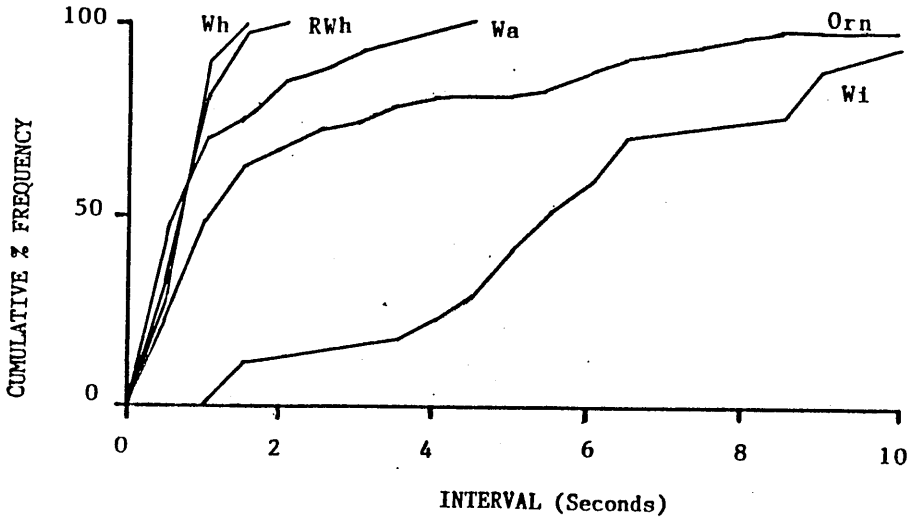


Figure 3.4 Latency from the first male action after a fan bout to his uninterrupted succeeding action.

Analysis of female behaviour.

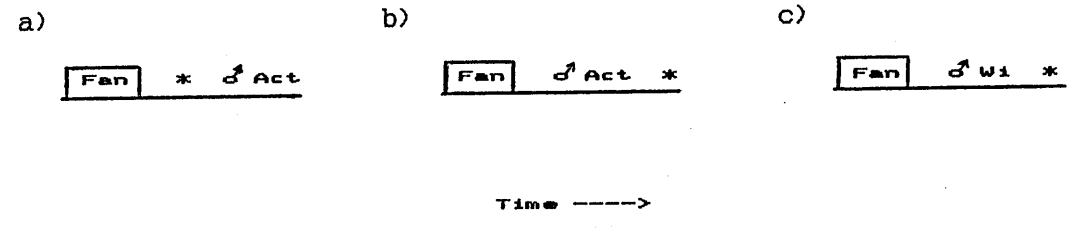
This section investigates the hypothesis that the wiggle influences the outcome of the female's behaviour by comparing the behaviour that a female would perform prior to the second male action after the end of a fan bout where there is no intervening wiggle, with that in the corresponding transition where an intervening wiggle is performed. Positive orientation acts (e.g. Oh+, +Adv) were performed by the female after 55.1% of all wiggles (n=216), but due to insufficient data involving other wiggle transitions, only those female behaviours associated with fan bouts are included here. Before the investigation can proceed however, it is necessary to clarify the limitations placed on analysis by the timing of the male's behaviour.

First, the interval between a wiggle and the preceding male act (FSt) is usually insufficient for a female to perform an interrupting action, hence analysis of the sequential transition of the female's movements is taken from within a fan bout, as this is the nearest point prior to a wiggle that the female generally has time to perform an interrupting action. Secondly, the data indicate that there is adequate time available for a female to perform a movement in the interval between a wiggle and the subsequent male action, therefore analysis of the outcome of female behaviour has only been extended to that occurring prior to the second male action after the fan bout has ended. Thirdly, where there is no wiggle, the variability in the temporal spacing of male actions following the end of a fan bout may present the female with two opportunities to complete a transition from within a fan bout and both must be considered when attempting to determine the sequential outcome of how a female may behave in the absence of a wiggle. These are in the interval leading to the first male action and in the interval between the first and second male action; both are dependant on an adequate interval to permit female movement, which may occur during one, both or neither of these male transitions.

The probability of a female transition from a positive, negative or unresponsive interrupt condition during a fan bout to the same interrupt conditions during the following first (no Wi) and second (no prior Wi, prior Wi) male transitions are shown in Table 3.4a-c, respectively. In line with earlier procedures, transitions involving advance behaviour have been omitted. The data in Table 3.4a and 3.4b are remarkably similar, with a high probability of female stasis between male transitions. This in itself is not surprising, given the information in Figures 3.3 and 3.4 which strongly indicates that many of the most commonly performed male

FEMALE BEHAVIOUR DURING PRECEDING FAN BOUT	INTERRUPTING FEMALE BEHAVIOUR % Transitions -ve	un	+ve	N. TRANSITIONS
a) *After FSt but before the male's 1st act (other than a wiggle).*				
-ve	20.0	68.7	11.3	115
un	11.5	70.2	18.3	104
+ve	1.1	86.5	12.4	178
b) *After male's 1st act (other than a wiggle), but before his 2nd.*				
-ve	20.0	72.2	6.9	115
un	6.5	86.9	6.5	107
+ve	0.5	82.8	14.4	180
c) *After a wiggle, but before the male's 2nd act.*				
-ve	0.0	46.7	53.3	15
un	11.1	32.0	51.9	27
+ve	0.0	0.0	100.0	9

Table 3.4 Transitions in female behaviour following a fan bout with and without a suceeding wiggle. Abbreviations: FSt = fan stop; -ve = negatively interrupted; un = uninterrupted; +ve = positively interrupted. N.B. Transitions interrupted by female advance (Adv) have been omitted.



Schematic representation, where * = occurance of female behaviour in relation to male acts

transitions from the end of a fan bout to the first and second subsequent act which do not incorporate a wiggle are performed with very short latency. These data contrast with those obtained after wiggles were performed (Table 3.4c) where there is a relative decline in the proportions of negative and unresponsive female behaviour and a marked shift towards positive behaviour. That there should be an increase in the probability of female movement occurring correlates with observations that there is a long interval available for female movement to occur after a wiggle. What is more significant is the increase in positive female transitions, which would not be predicted on the basis of a longer interval alone. This suggests that the female tends to perform receptive behaviour in response to a wiggle.

It is possible that the probability of one of the three classes of female behaviour performed after the fan bout has ended is correlated with the occurrence of similar behaviour performed during the fan bout, but the data indicate that a strong general link of this nature does not exist where a wiggle is not involved. However, there does appear to be some reinforcement of earlier positive female behvaiour if a wiggle is subsequently performed.

To determine whether the performance of a wiggle after a fan bout has any influence on the timing of the female's behaviour, the latency to the first female movement following the end of a fan bout during which the female had remained static or had performed a negative head movement (the two most common female behaviours preceding a transition in male behaviour to the wiggle) were measured irrespective of the order of male transition during which that movement had occurred. [It must be stressed that there is no assumption at this point that the influence of the wiggle or any

other intervening male action is either constrained or enhanced by the performance of a second or subsequent male action, or that transitions in female behaviour are independent of earlier transitions.] The cumulative probability of the latency to female movement is shown in Figure 3.5 for fan bouts which were followed by a wiggle and those that were followed by any other male action. These data suggest that the performance of a wiggle after a fan bout does not appear to significantly influence the latency of female movement compared to that which would occur in the absence of a wiggle. Thus the results indicate that the wiggle influences the sequential but not temporal outcome of the female's behaviour, by increasing the probability of a transition from unresponsive or weakly unreceptive female behaviour to positive behaviour, rather than by bringing forward or delaying the transition in female behaviour which would otherwise occur.

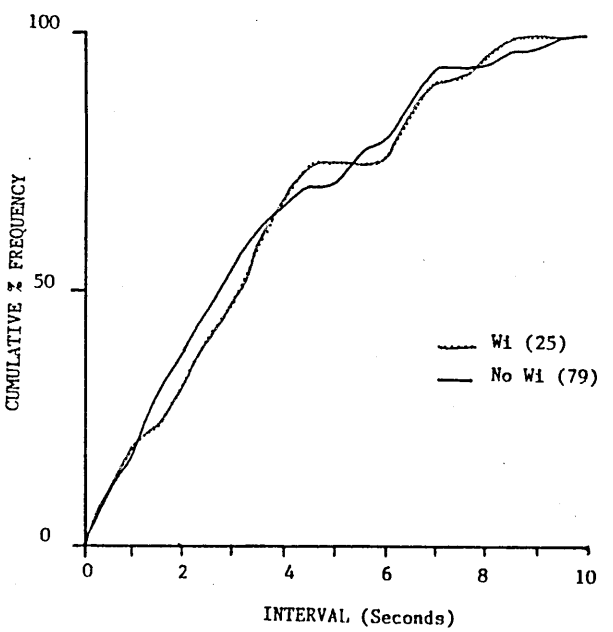


Figure 3.5 Cumulative probability of latency of female movement after a fan bout. Key: dotted line = male performs an intervening wiggle; continuous line = male performs an intervening action other than a wiggle. Figures in brackets = number of observations on which the plot is based.

Discussion

The results of these analyses indicate that the wiggle serves to increase the receptivity of females which are at least temporarily unreceptive and therefore, suggest that the primary function of the wiggle is one of persuasion. In what context may this be important? I suggest that the performance of the wiggle is of greater value at some stages of a courtship encounter than at others. For example, a series of positive movements by the female indicates that she is already receptive and therefore the male would gain little by attempting to increase her receptivity still further by performing a wiggle. Under these circumstances the male may benefit most by performing retreat display and ultimately spermatophore transfer behaviour. At the other extreme where the female moves away from the male, her position would not allow her to see the male's wiggling tail tip. It would therefore be of most benefit to the male if he regained his display position in front of the female by performing an orientation act such as a move-to-the-front.

Unresponsive and weakly unreceptive female behaviour are both conditions in which it is still possible for the female to see a wiggle display being performed. These female behaviours generally occur during two stages of an encounter - near the start when the male begins to overcome the female's initial tendency to move away and towards the end (usually after an earlier stage of receptive behaviour), but before the female begins to move away. Performing a wiggle during the former stage may act to increase a female's receptivity, whilst performing one in the latter stage may act to partially restore a female's earlier level of receptivity. Performing the wiggle where there is the beginning of a potential transition in female receptivity from negative to positive or from

positive to negative, may also contribute to increasing the probability of a further spermatophore being deposited.

It is impossible to determine how long the influence of the wiggle remains with the female, but observations show that in 65.6% of the 32 courtship encounters in which wiggles were observed, the male was subsequently successful in achieving spermatophore deposition after further courtship. This can be compared to 16.7% success to deposition in the six courtship encounters in which wiggles were not performed. Logically, any increase in the sexual receptivity of a female elicited by the performance of a wiggle (if maintained or developed by further courtship to the point where the transfer of sperm and subsequent insemination of the female occurs) which may otherwise not have been achieved, would clearly be adaptive for the male.

The data suggest that the wiggle is a fairly effective mechanism for re-gaining the attention of the female, but what is the nature of the stimulus to which she responds? To human eyes the movements of the tail during this act are similar to the physical motion of aquatic arthropod larvae. It is therefore possible that the wiggle provides a stimulus of movement which a female interprets as prey and hence, that it functions as a prey mimic which is used by a male to lure females. Evidence to support this is provided by anecdotal observations of a few females lunging towards a wiggling tail tip in a similar manner to that observed during feeding (Joly 1981). However, no females attempted to bite a wiggling tail, although this has been observed in *T. vittatus* (Raxworthy 1989b). This may be because the male ceases movement and quickly reverts to display in response to a positive movement by his partner. If the wiggle acts as a prey mimic, then its relatively conservative use may both prevent the male

from being bitten and also delay any learning processes which may limit its effectiveness. Without further empirical investigation the exact nature of the stimulation provided by the wiggle must remain open to speculation.

VARIATION IN BEHAVIOUR DURING T. H. HELVETICUS COURTSHIP ENCOUNTERS.

Before a meaningful comparison can be made between the courtship behaviour of *T. helveticus* with newts of other species, it is first important to analyse the natural variation that occurs during a conspecific *T. helveticus* courtship encounter. Such variation may include qualitative and quantitative changes in the performance of specific display movements, or in the timing or order of those movements. These factors may provide clues to the significance of display actions during courtship. This section investigates the variation that may occur:-
1) within the phases of an encounter (orientation, static display, etc.) and 2) between the progressive sequences of an encounter.

Method

Data were obtained from the 31 timed courtship encounters obtained earlier and a further 30 untimed courtship encounters recorded as a spoken commentary, carried out under otherwise similar conditions. During the earliest of the timed recordings, the length of an object in the aquarium (e.g. a flat stone) was used as a scale for measuring the distance between the snouts of partners during fan bouts, but this was later replaced by a $5cm^2$ grid overlaid on the substrate. Fan angles were measured as shown in Figure 3.6; they were copied directly off the video monitor screen onto acetate sheets. Intersnout distances before correction for scale differences were measured using the same method. As the edges of the aquarium occasionally restricted tha angle that males adopted during the

fan display, these and other distorted fan stances (such as those performed when not on the substrate) were omitted from further analyses.

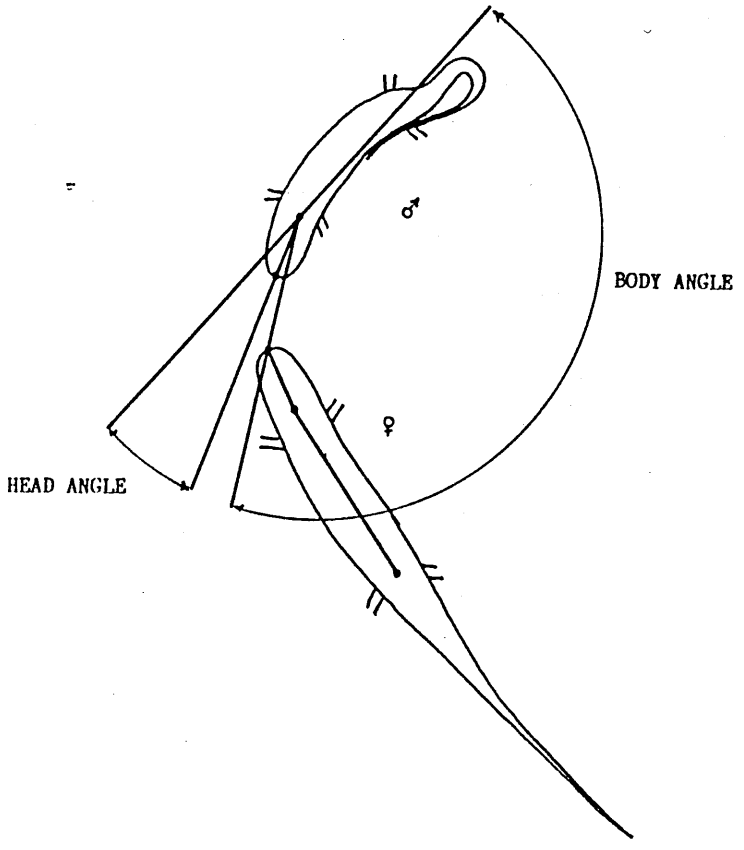


Figure 3.6 Measurement of fan angles.

Aspects of behaviour within the four phases of courtship which merited analysis included the behaviour of the male at the start of an encounter, variation in the performance of fanning display (angle and duration of fanning and intersnout distance), variation in the proportion of static (Wa, Wh, F, Wi) and retreat display (RWa, RWh, RF) acts, variation in the frequency of some spermatophore transfer acts (Pb, TT) and the success of spermatophore pick-up (S↑) during an encounter. As some of these parameters appeared not to follow normal distributions (e.g. F duration), non-parametric descriptive statistics have been used.

Analysis of variation between sequences has been confined to the first three complete sequences and any incomplete sequences due to low sample sizes of fourth and subsequent complete sequences. Two categories of incomplete sequence are recognised, based on whether prior spermatophore deposition has taken place. Those which occur at the start of an encounter (i.e. no prior S↓) have been named 'only' sequences (O), whilst those that occur at the end (i.e. after prior S↓) are named 'residual' sequences (Res). The three successive complete sequences and the corresponding transfer phases have been named '1st', '2nd' and '3rd', respectively.

The duration of the intervals between potential spermatophore transfer phases in which the male has an opportunity to display (display time - defined as the interval between the start of a sequence and the start of a spermatophore transfer phase for the complete sequences and the whole of a sequence for the incomplete sequences), the rate of Sn, M, Wa, WH, RWh, F and RF (some fanning bouts have both a static and retreat component and therefore the two categories cannot be meaningfully separated) within display time and the proportion of display time spent fanning, were calculated and compared between sequence groups from the 31 timed encounters. The duration of successive spermatophore transfer periods (transfer time - defined as the interval between the creep and the last act of spermatophore transfer, usually Pb) were calculated together with the number of tail touches performed prior to S↓, the number of Pb after braking and the success of spermatophore transfer. Non-parametric statistical tests (Mann-Whitney U Test, two-tailed) were used in view of the limited sample sizes of some sequences (O: n=14, 1st: n=17, 2nd: n=13, 3rd: n=7, Res: n=17 for all acts except Wi). Wiggles were recognised as a separate act in the last eight of the timed encounters and as a

consequence of low sample sizes (O: n=2, 1st: n=6, 2nd: n=5, 3rd: n=4, Res: n=6), the rate of Wi during O sequences could not be statistically tested.

Variation within display phases.

a) The behaviour of the male at the start of an encounter.

A courtship encounter does not always commence as a consequence of a chance meeting in the aquarium. On occasions the male may approach a moving female directly, adopting the alert posture (AP) before moving in short bounds towards his partner from some distance away. When the male is within a few centimetres of the female he is equally likley to sniff her, or to begin to display immediately (Table 3.5). A move-to-the-front is most frequently the first display act performed after sniffing.

Sniffing First 41.7%				χ^2 Test n=60, χ^2=1.35, 1df P>0.05	Courtship Display First 58.3%			
1st Display Act After Sniffing n=25					1st Display Act, No Sniffing n=35			
M	Wa	Wh	F		M	Wa	Wh	F
52%	12%	28%	8%		26%	23%	17%	34%

Table 3.5 Male behaviour near the start of a courtship encounter.

b) Variation in fanning behaviour.

Including periods of retreat and fan, which may occur during a small part or during all of a fanning bout if it is very short, the male spends a mean of 27.8s/min performing fanning display. The duration of continuous fanning bouts lasted between 0.1s and 486.6s, median 7.2s (n=1836). The typical stance adopted by the male is shown in Figure 3.6. Where access to the female is not obstructed, the male positions himself so that the median distance between the snouts of the partners is 13.0mm (range 3-

72mm, n=612), with his head at a median angle of 10° (range 0°-54°, n=689) to the midline between his neck and pelvic girdle; and his body at a median angle of 145° (range 50°-180°, n=693) to her snout. Male newts do not seem to have a preference for fanning on either side and will turnabout so that the tail nearly always beats on the side facing the female (Finkler 1923).

c) Variation in the relative proportion of static and retreat display acts. There is considerable variation in the relative proportion of static and retreat display acts performed (Figure 3.7). Fan bouts and RWh are the two most common acts performed during static and retreat phases, respectively. Although both F and Wa are clearly important display movements, RF and RWa are relatively rare.

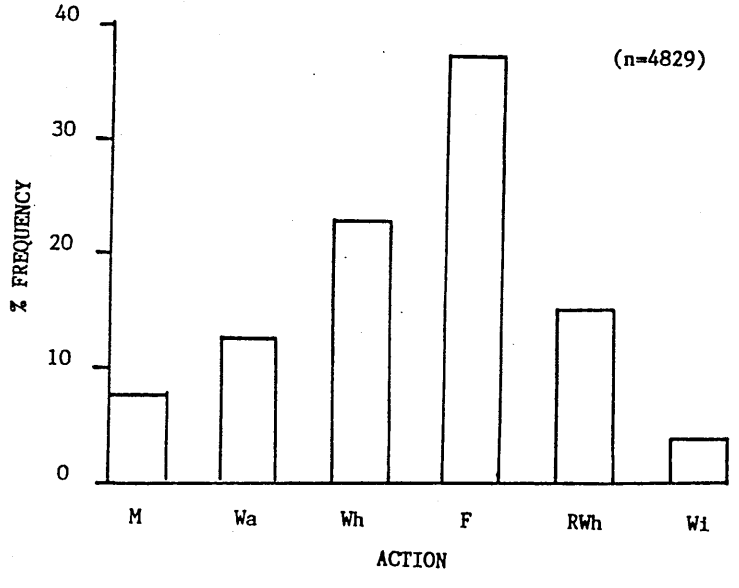


Figure 3.7 Proportion of static and retreat display acts performed by male *T. h. helveticus*. RWa and RF are too few to include.

d) Variation during the spermatophore transfer phase.

The male frequently varies both the direction and the duration of creep behaviour and sometimes this occurs when the aquarium wall forms an obstruction to his initial line of movement. Once the male begins to quiver, the female may perform up to three tail touches before a spermatophore is extruded, but a median of one is normally performed (n=40) and occasionally deposition occurs in the absence of a tail touch. After spermatophore deposition has taken place and the male has turned into the braking position, the female gives a median of one tail touch before a push-back is performed, but up to two tail touches have been observed (n=150 Pb). Most commonly there is only one push-back exchange, but up to five may be performed (n=102 Br). Push-back resulted in the subsequent pick-up of a spermatophore which would otherwise have been missed in two (3.1%) spermatophore transfer phases where there was no intial pick-up (n=64).

The spermatophore does not always adhere to the female's cloaca and was missed during more than 39 courtship encounters involving over 104 spermatophore transfer phases for one of the following reasons:-

* The female's body does not travel in the correct line towards the spermatophore and consequently, the central part of the cloaca and the spermatophore do not come into contact (the most common cause). Sometimes this results in the spermatophore adhering to another part of the female's body (e.g. her foot).

* The male does not move far enough forward when creeping-on, resulting in the female moving an inadequate distance forward for her cloaca to reach the spermatophore.

* The spermatophore may not be released properly by the male and so either attaches to his cloaca, or is deposited away from the site of extrusion (observed several times in one male only).

* The spermatophore may not be deposited at all (observed only once).

The latter two cases are probably a consequence of defective spermatophore production (i.e. lacking caps or bases: Halliday & Waights, pers. comm.).

In 63.9% of all courthip encounters in the laboratory the male produced between one and six (median three) spermatophores (Figure 3.8) and out of the total of 104 deposited, 40.4% were picked-up. The probability of at least one successful pick-up increased during an encounter with the number of spermatophores deposited and out of the 39 encounters in which deposition took place (Table 3.6), 69.2% resulted in at least one of the spermatophores being successfully transferred to the female.

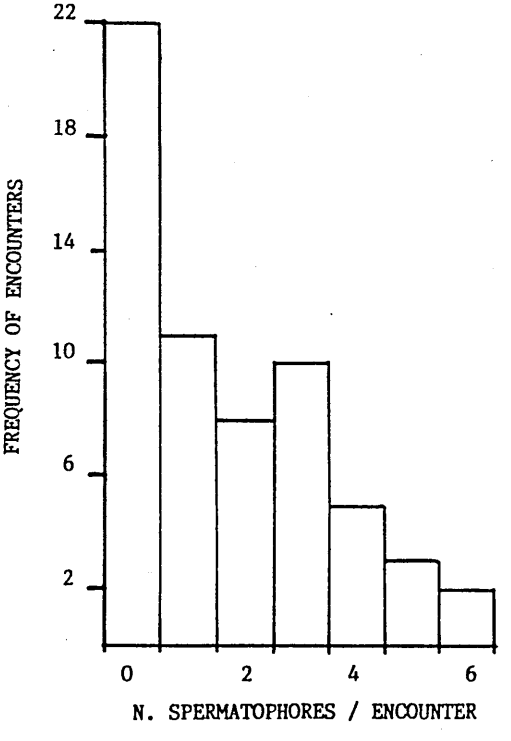


Figure 3.8 Number of spermatophores deposited during an encounter.

	NUMBER OF SPERMATOPHORES DEPOSITED						
	1	2	3	4	5	6	Total
Total encounters	11	8	10	5	3	2	39
Successful encounters	5	6	7	5	2	2	27
% Successful	45.4	75.0	70.0	100.0	66.0	100.0	69.2

Table 3.6 Pick-up success during encounters. An encounter is defined as successful if there is at least one pick-up within its duration.

Variation between sequences.

a) The duration of sequences.

The range of display time in each sequence group was highly variable, but less so for transfer time (Table 3.7). Six (19.3%) of the display durations in the incomplete sequences were longer than those for any of the complete sequences, yet they did not culminate in spermatophore transfer. A significant decrease in the duration of display time occurred between the 1st and 2nd sequences, but no difference was found between any of the other groups tested. In contrast, there was no significant difference in the duration of transfer time between the 1st and 2nd sequence, but that of the 3rd sequence was longer than that of the 2nd.

b) Variation in the rate and duration of acts during display time.

The median rate (±95% Confidence Limits) of Sn, M, Wa & RWa, Wh, F & RF, Wi and RWh and the % display time spent fanning, are shown in Figures 3.9 and 3.10 and the results of Mann-Whitney U Tests between the sequences groups are shown in Table 3.8. The highest rates of fanning were found at the start (O, 1st) and end (Res) of an encounter, but sniffing was relatively uncommon in the other complete sequences (2nd, 3rd). Although the rates of move-to-the-front followed similar trends, the only significant variation in the rate of performance of this act occurred between the 3rd and residual sequence groups, when a marked increase was

	SEQUENCE	DURATION Median	±95% Conf. Limits	N. Sequences
DISPLAY TIME	0	404.5s	286.1 - 805.6s	14
	1	302.8s	169.3 - 597.4s	17
	2	93.9s	18.0 - 253.3s	13
	3	266.3s	154.9 - 445.0s	7
	Res	478.9s	199.6 - 896.5s	17
TRANSFER TIME	1	42.9s	31.5 - 51.1s	17
	2	41.0s	32.1 - 59.6s	13
	3	71.3s	53.0 - 103.5s	7

Table 3.7 Total duration of display time and spermatophore transfer phases during different sequences. The only significant differences between sequences were: Mann-Whitney U Test - Display Time: 1st & 2nd sequence, $U=44.5$, $P<0.05$; Transfer Time: 2nd & 3rd sequence, $U=16.0$, $P<0.05$.

observed. Thus the male performs move-to-the-fronts more often towards the end of an encounter with a female that has once been sexually responsive than in the early stages of the encounter. This contrasts with the trends found for waves and whip rates, which did not alter significantly between any of the sequence groups. The two aspects of fanning display investigated here (i.e. rate and % time spent fanning) did not follow the same pattern. The following results were found:-

* The male spends a larger proportion of display time engaged in fanning in sequences that lead to spermatophore transfer (1st, 2nd, 3rd) than in those that did not (0, Res).
* There were no significant differences in the proportion of display time spent fanning between any of the three complete sequences, or either of the two incomplete sequences.
* There is an increase in the rate of fan bouts from the 1st to the 2nd sequence and this increased level is maintained in subsequent (3rd, Res) sequences.

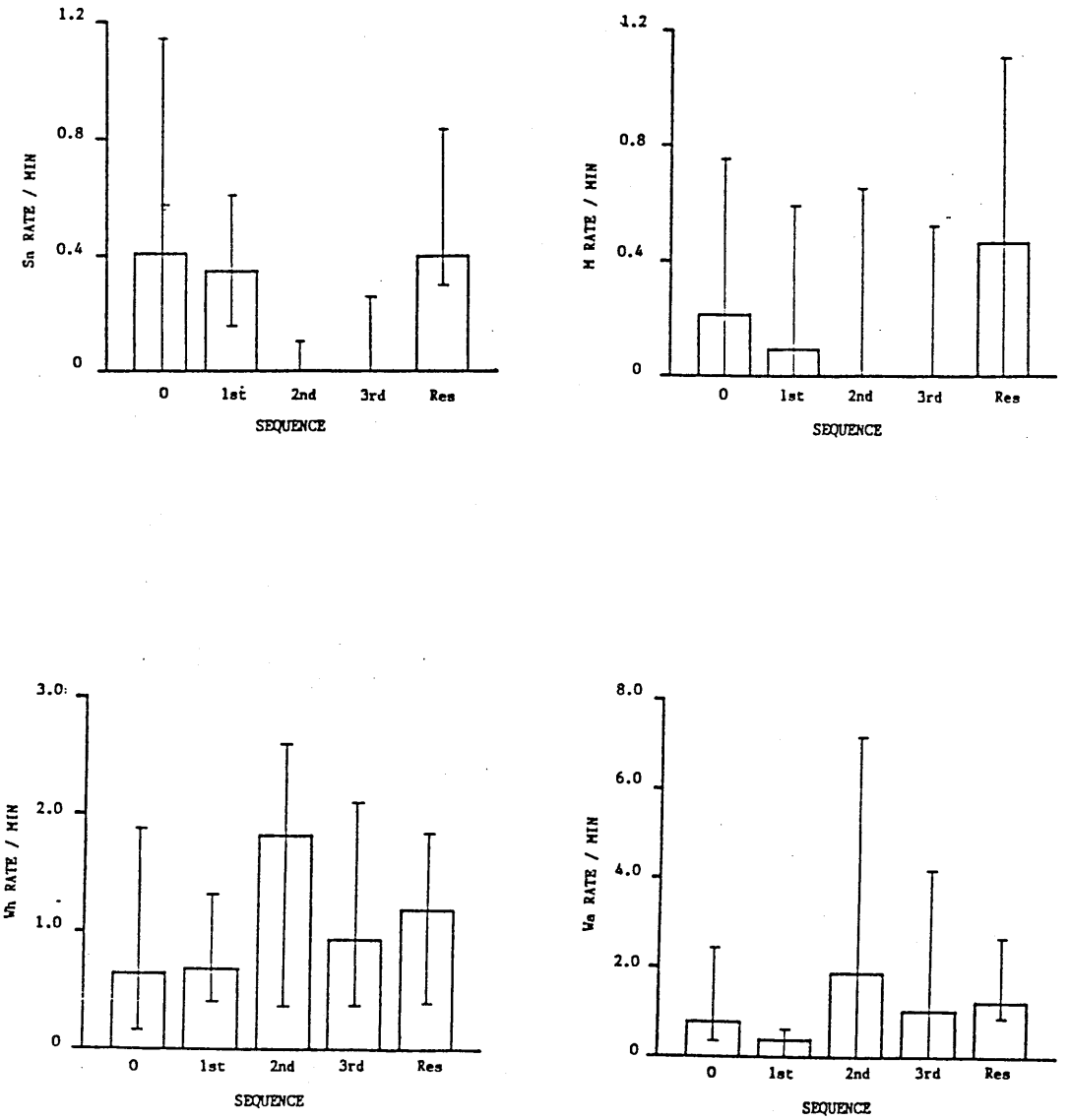


Figure 3.9 Variation in male behaviour between sequences (I). Key to male *T. h. helveticus* acts: Sn = Sniffing; M = Move-to-the-front; Wh = Whip; Wa = Wave. Histograms represent median values, bars indicate 95% Confidence Limits.

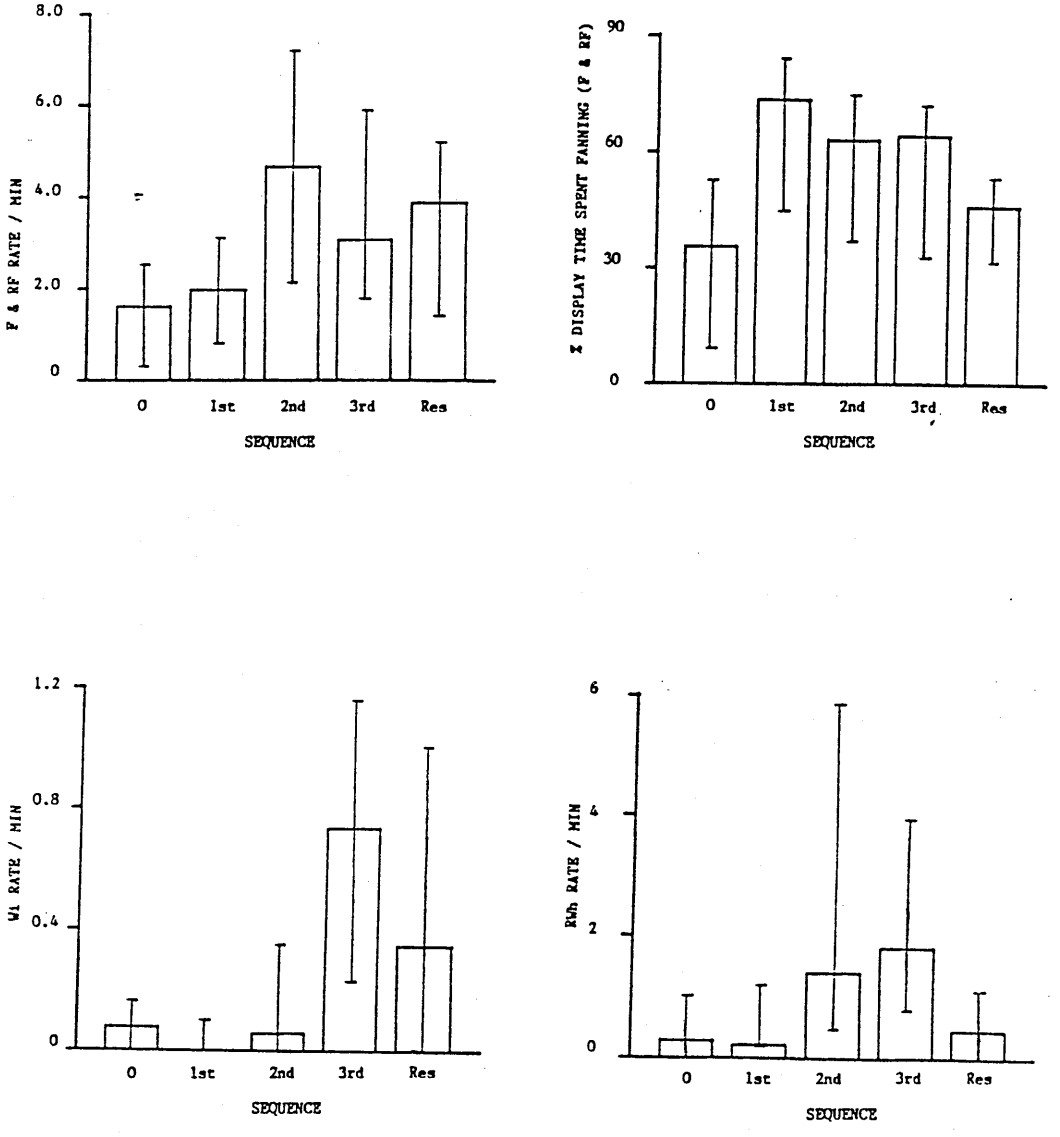


Figure 3.10 Variation in male behaviour between sequences (II). Key to male *T. h. helveticus* acts: F & RF = Fan and Retreat & Fan; Wi = Wiggle; RWh = Retreat & Whip. Histograms represent median values, bars indicate 95% Confidence Limits.

SEQUENCES COMPARED	M-W	MALE BEHAVIOUR Sn rate	M rate	Wa rate	Wh rate	F rate	% DT F	Wi rate	RWh rate
0 & 1st	n	14,17	14,17	14,17	14,17	14,17	14,17	2,6	14,17
	U	110.0	98.5	91.0	112.5	91.0	56.0	4.0	103.0
	P	NS	NS	NS	NS	NS	<0.05	IS	NS
1st & 2nd	n	17,13	17,13	17,13	17,13	17,13	17,13	6,5	17,13
	U	39.0	101.0	70.5	71.5	42.5	90.0	20.0	71.0
	P	<0.05	NS	NS	NS	<0.05	NS	NS	NS
2nd & 3rd	n	13,7	13,7	13,7	13,7	13,7	13,7	5,4	13,7
	U	43.0	38.0	37.5	36.0	29.0	45.0	1.0	37.0
	P	NS	NS	NS	NS	NS	NS	<0.05	NS
1st & 3rd	n	17,7	17,7	17,7	17,7	17,7	17,7	6,4	17,7
	U	22.0	42.0	40.5	42.5	29.0	48.0	0.0	18.0
	P	<0.05	NS	NS	NS	NS	NS	<0.05	<0.05
3rd & Res	n	7,17	7,17	7,17	7,17	7,17	7,17	4,6	7,17
	U	15.5	27.5	53.0	59.5	56.0	23.0	6.0	17.0
	P	<0.05	<0.05	NS	NS	NS	<0.05	NS	<0.05
0 & Res	n	14,17	14,17	14,17	14,17	14,17	14,17	2,6	14,17
	U	114.5	83.0	89.0	86.0	57.0	99.0	1.5	111.5
	P	NS	NS	NS	NS	<0.05	NS	IS	NS

Table 3.8 Comparison of male behaviours between sequences. Abbreviations: M-W = Mann-Whitney U Test (two-tailed); NS = >0.0f; IS = insufficient sample size; % Dt F = % Display time spent fanning; for others see Table 3.1.

Wiggles are performed proportionately more often in the later sequences (3rd) of an encounter after spermatophore deposition has occurred than in the earliest complete sequences (1st & 2nd) where wiggling is relatively rare. Wiggle rates do not follow the same trends as any of the other aspects of fan display investigated here, which indicates that the factors which influence fanning due not wholly influence wiggle rates. Wiggles most closely follow the same trends as retreat and whip, which are performed progressively more often from the 1st to the 3rd sequence and less so in each of the incomplete sequences.

SEQUENCE	N. SEQUENCES	NUMBER OF TAIL TOUCHES median	range	MANN-WHITNEY U TEST
1st	17	1	0 - 3	1st & 2nd: U=106.5, P>0.05
2nd	13	1	1 - 2	2nd & 3rd: U=40, P>0.05
3rd	7	1	1 - 2	

Table 3.9 Number of tail touches prior to spermatophore deposition in successive sequences.

c) Variation between sequences during transfer time.

During the spermatophore transfer phases there was no significant difference in the number of tail touches performed prior to deposition between sequences (Table 3.9), but a progressive decline in pick-up success from 51.3% to 20.0% was observed between the 1st and 3rd transfer phases (Table 3.10). The median number of push-backs increased from two to three from the 1st to the 2nd sequence; up to five push-backs occurred in the 3rd (Figure 3.11).

	SEQUENCE 1st	2nd	3rd
Spermatophores Deposited	39	28	20
Spermatophores Picked-up	20	12	4
Pick-up Success (%)	51.3	42.9	20.0

Table 3.10 Success of spermatophore pick-up in successive sequences.

Discussion

Although a male may often commence an encounter by sniffing his partner, this act is clearly not an essential pre-requisite to spermatophore transfer and consequently, it must be assumed that the primary function of sniffing is linked to either mate assessment of the male, or stimulation of the male, but not for stimulation of the female. Assessing the value of

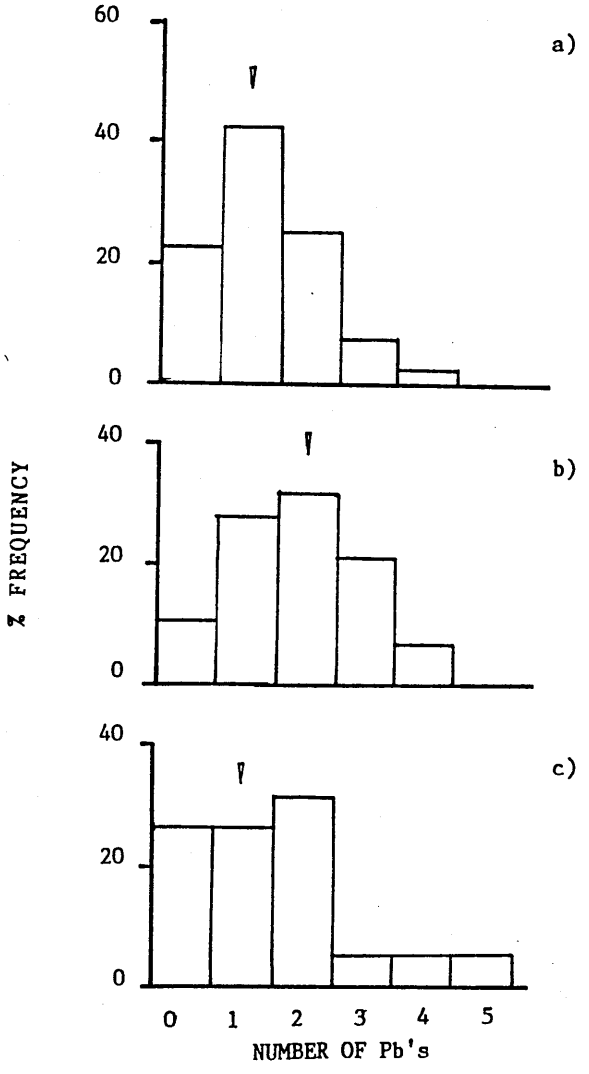


Figure 3.11 Variation in number of push-backs between *T. h. helveticus* sequences. Marker denotes median value. a) = 1st sequence, b) = 2nd sequence, c) = 3rd sequence.

a partner would presumably have greatest value to a male if carried out in the earliest sequence of an encounter and this may account for the reduced rate of sniffing in the intervals between successive transfer phases. Halliday (1977a) suggested that sniffing provides the male with information relating to a female's reproductive state or her oocyte compliment; these suggestions are given some support by Verrell's (1986b) demonstration that male *T. vulgaris* show a preference for larger, more fecund females when presented with olfactory cues alone. Sniffing may also act as a mechanism for conspecific mate recognition and the prevelance of this act in residual sequences also suggests that it is used to assess whether a female has been successfully inseminated. If female receptivity declines with the number of spermatophores picked-up, then it may be assumed that such a mechanism may be adaptive for the male by enabling him to conserve resources which would otherwise be unnecessarily expended during further courtship of an unreceptive partner.

The predominance of fanning during *T. helveticus* courtship in this study suggests that it is one of the most important acts in the courtship repertoire of this species. It probably enables transfer of sensory stimulation to take place via three modalities (olfactory, visual, tactile) simultaneously. The factors that influence whether a male is successful in persuading a female to mate at the start of an encounter appear to be predominantly related to the proportion of display time spent fanning and not the duration in which a male engages a female in courtship, or the rate at which courtship acts are performed. Therefore, it is probably during the relatively long early fan bouts that the primary external stimulation responsible for bringing about a positive motivational state in the female is delivered. Move-to-the-fronts and whips probably play a preliminary role in this change by reducing the female's early tendencies

to flee. In contrast to the whip (where a transient but relatively forceful blast of water is produced), during the fan the male adopts a characteristic position which allows a sustained directional stream of water to be channelled towards the female's head where the olfactory nares and a large proportion of the lateral line organs are situated (Smith 1973). By varying the angle of the body (and/or the head) during fanning, the male is able to alter the direction of the water stream. This would improve the efficency of transfer of vibrational signals and of chemicals produced from his cloacal region. The fan is therefore more efficient as a mechanism for the transfer of chemicals than any other courtship act.

Many of the differences observed between successive sequences are probably largly due to the changing motivational state of the partners, brought about by an interaction between internal factors and external stimuli. At the start of an encounter, a prolonged period of courtship display is usually required to overcome a female's initially unreceptive state, but this period of display is reduced in the period between the first and second spermatophore transfer phases. This suggests that the sexual motivation of both partners remains high at this stage. In females this may be due to a combination of the accumulation of stimulation from earlier male display carried over the relativly short first transfer phase, and because any inhibitory effects relating to pick-up (if they exist) may not have had sufficient time to adversely affect the female's internal state. If the male has the capacity to deposit further spermatophores, then a short display period would conserve his oxygen supply for later sequences. Hence, the male's level of sexual motivation may also be maintained as a consequence of favourable internal factors. A high proportion of respiritory organs are contained within the skin (Czopeck 1959) and although the male supplements the reserve of oxygen held in the

lungs by absorbing oxygen directly from the water (Foxon 1964, Ultsch 1976), it must be assumed that conservation of oxygen must be important to males, as courtship involves a high level of sustained activity and general activity is correlated with the frequency with which newts make breathing ascents (Halliday & Worsnop 1976). In addition, ascents for air are commonly associated with courtship display (Halliday & Sweatman 1976) and it is probable under natural conditions, that a male that leaves a female during a courtship encounter to ascend for air will be unlikely to find his partner again on return to the substrate (Halliday 1977b).

Once the encounter progresses to the 3rd sequence, the male performs the wiggle at a higher rate, which earlier analysis indicates is in response to slightly unreceptive female behaviour and which in turn suggests that the female's motivational level has begun to decrease at this stage. Because wiggles are performed predominantly in the later sequences of an encounter and because thay are an act that requires relatively little movement and presumably, energy expenditure, it must be questioned whether wiggles are performed in part, as a consequence of male fatigue. If this were so then it would be predicted that during the same period there would be a decrease in the proportion of the time spent fanning, as fanning is probably the most energetically expensive display act and it is also the male act most commonly associated with the wiggle. The high proportion of display time spent fanning in the earlier sequences did not decrease significantly in the 3rd sequence, hence these results suggest that wiggles are unlikely to be performed as a direct consequence of male fatigue.

The increase in the rate of retreat and whips between complete sequences may be a mechanism by which a male may 'test' a female's continued receptivity. By only depositing further spermatophores to females

responding with sustained positive behaviour, he may be avoiding depositing spermatophores to a female that is unlikely to complete the full repertoire of transfer phase responses, thereby conserving spermatophores for future encounters. This would be adaptive, as a latency of 48 hours is required before a male is again able to produce his full complement of spermatophores (Verrell 1986a). As the male does not require an increase in the number of tail touches as the encounter progresses before he extrudes a spermatophore, this suggests that the tail touch is probably a highly reliable indicator of female responsiveness in this species.

Variation in other behaviour patterns may function to increase the efficiency of spermatophphore transfer. By varying the distance over which he creeps, the male may be facilitating pick-up by selecting the most suitable substrate in his immediate vicinity on which to deposit a spermatophore. Also, the creep, quiver and creep-on may be mechanisms by which the male improves the alignment of the female to increase the probability that a spermatophore will come into contact with her cloaca and, by requiring a tail touch from the female before the spermatophore is deposited, the male may confirm the female's continued presence and receptivity even when he has turned away from the female and is no longer able to gain such information from visual cues. The performance of push-backs increases the probability of pick-up, which clearly increases the efficiency of spermatophore transfer. It is interesting that male *T. helveticus* will frequenctly perform a push-back without a tail touch and it may be that in good lighting conditions the visual cues available to a male are adequate to evoke this response, but that in poor lighting conditions, such as when courting at night, the tactile stimulation provided by a tail touch gains in significance.

Although the probability of achieving insemination increases with the number of spermatophores deposited during an encounter, the retention of spermatophores which the male has the potential to deposit immediately (and which otherwise would be wasted) holds several advantages, assuming that he would have further opportunities for mating with other females. Primarily, it would conserve his limited spermatphore supply (Verrell 1986a), thus allowing him to mate with a greater number of females over a breeding season and secondarily, such spermatophores would have a higher probability of pick-up if they were the first to be deposited during a subsequent encounter, rather than if they were deposited in later sequences of an existing encounter.

It is not known why *T. h. helveticus* spermatophores are less likely to be picked up as the encounter progresses. The data suggest that the female increasingly follows an inappropriate line of movement, but as yet there are no directly identifiable factors in either male or female behaviour to indicate why this should occur. Alternatively, this phenomenon may arise as a consequence of limited male resources. By producing successively smaller quantities of either spermatozoa, or the medium in which they are deposited, the male may extrude progressively smaller spermatophores, which are more likely to be missed than larger spermatophores. Increasing the frequency of push-backs as the encounter progresses may therefore be a mechanism that has evolved in males to provide some compensation for this decreasingly efficient pick-up process; the longer duration of transfer time in the 3rd sequence may be partly explained by the incorporation of these additional push-backs. Interestingly, the probability of pick-up also decreases with sucessive *T. vittatus* spermatophores (Raxworthy 1989b), but it increases in *T. v. vulgaris* (Halliday 1974) and *T. montandoni* (Pecio & Rafinski 1985).

Extending transfer time in later sequences may convey additional benefits, such as allowing the female more time to follow the correct path, or the male to select a better site for deposition. However, the former hypothesis is not supported by the analysis of pick-up in the preceding two sequences (1st & 2nd) where, despite their shorter duration, pick-up success is higher. Similarly, the second hypothesis would also seem to be improbable, as a male would presumably gain little by delaying a sequence solely to select a marginally better extrusion site in an aquarium with a relatively uniform substrate. However, due to the limitations to free movement imposed by the walls of the aquarium, these two hypotheses cannot be totally discounted as occuring in the wild. It is more likely that the variation in the duration of transfer time in the 3rd sequence reflects the constraints places upon the male by a combination of decreasing female receptivity and his limited oxygen and spermatophore supplies.

A COMPARISON OF THE COURTSHIP BEHAVIOUR OF T. V. VULGARIS AND T. H. HELVETICUS.

I have already identified a number of sensory modalities which may be stimulated by courtship display and have suggested that any cues produced may vary in prominence according to both the catagory of behaviour and the stage of courtship during which they are performed. If elements of the male's courtship repertoire provide the cues which prevent or reduce interspecific matings, then recognition of these cues must occur before spermatophore transfer is completed, most probably in the initial stages of an encounter (Liley 1966) prior to the period of sustained female approach that indicates sexual receptivity. The courtship displays of *T. vulgaris* and *T. helveticus* follow broadly similar patterns, but vary most in the static display phase. Differences between the species in this early

stage may constitute an integral part of the isolating mechanism by providing the cues on which species selectivity is based; four groups of potential variation are discussed in this section.

Specific stimuli.

Apart from the right angle tail hold observed by Raxworthy (1989a) during *T. v. vulgaris* courtship, there are no other reports of actions performed by one sex during *T. vulgaris* and *T. helveticus* courtship that is not also performed by their heterospecifc counterpart, although some entirely different acts are described in other *Triturus* species (eg. Green 1989, Giacoma & Sparreboom 1987, Pecio & Rafinski 1985, Raxworthy 1989b). Even the *T. helveticus* wiggle is not unique, as a homologous act has been observed in *T. vulgaris* encounters (Raxworthy 1989a, Wambreuse & Bels 1984). This does not mean however, that species-specific stimuli are absent.

Based on the differences in form, colour and patterning between both male and female morphology observed in *T. vulgaris* and *T. helveticus* in Chapter 2, visual cues may be amongst the first to operate, even before courtship has commenced. Similarly, if species-specific odours are produced by either sex, then these olfactory cues may be detectable by both species, either in the water from a relative distance, directly whilst sniffing, or during courtship. During sniffing, males of both species frequently touch the female's head, trunk, cloaca or tail and so it cannot be discounted that chemical information is transferred directly to the female, as it is in other urodeles where close physical contact occurs. In the Red-Spotted newt (*Notophthalmus viridescens*), glandular secretions are applied to the female's nares (Arnold 1977) during amplexus, whilst in Plethodontid salamanders, the male rubs his mental gland on parts of the female's body.

In some other species such as *Desmognathus fuscus*, the male takes this a step further by 'inoculating' the female with his secretions after first abrading her skin with specialised premaxillary teeth (Arnold & Houck 1982, Houck & Reagan 1990). As male *T. vulgaris* and male *T. helveticus* are not thought to have a specialist secretory gland situated in the snout area any chemical cues transferred in this way would probably originate from normal skin secretions.

Differences in the form of display acts.

Recent comparisons of the form of display acts performed by male *T. v. vulgaris* and male *T. helveticus* have been carried out by Halliday (1972), Raxworthy (1989a) and Wambreuse and Bels (1984). Neither reports by these authors, nor my own observations, suggest any distinction between the movements carried out during move-to-the-front. The form of waves varies considerably within these two species and therefore it is difficult to make a valid interspecific comparison for this act. However, in whips, there appear to be some differences: the tip of the tail is held closer to the body in *T. helveticus* than in *T. vulgaris* and in the latter species the whip is altogether a more forceful movement which is frequently carried out with such vigour that the distal portion of the male's tail strikes the female (Raxworthy 1989a). This is an unusual event during *T. helveticus* whips.

Some qualitative differences are reported to exist in the stance adopted by males during both the wiggle and the fan. Wambreuse and Bels (1984) report that male *T. vulgaris* perform the wiggle with the tail tip held slightly further away from the flank and at a reduced angle (about 45°) compared to male *T. helveticus*, but Raxworthy (1989a) disputes their observation and reports that no such differences exist. However, striking

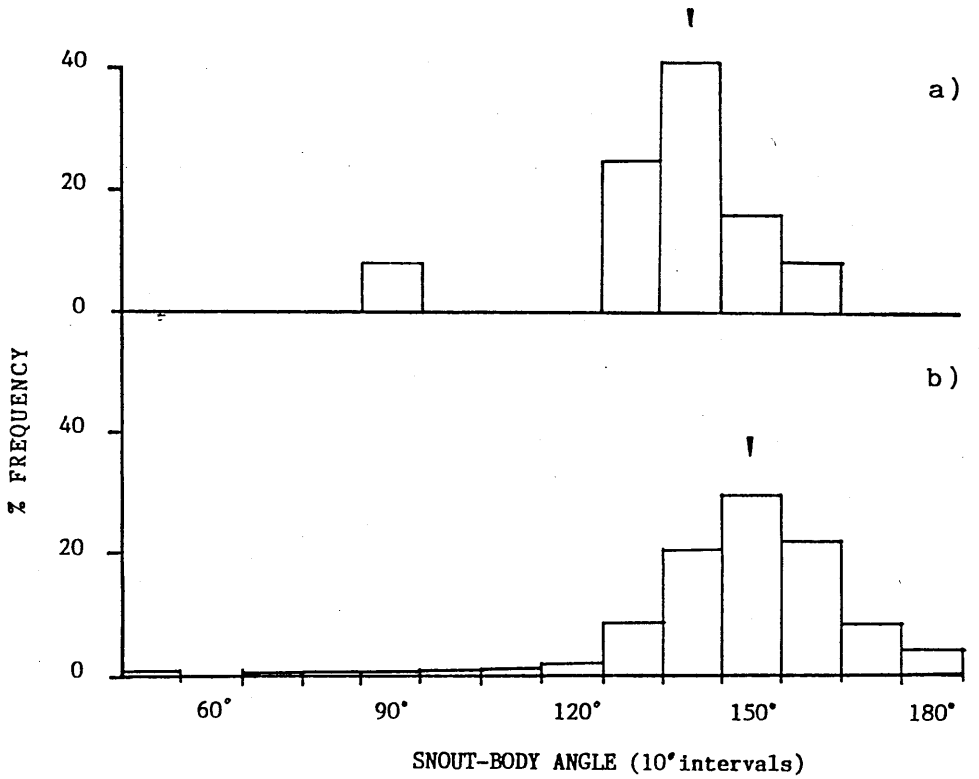


Figure 3.12 Angle between male's body and female's snout during fan bouts. a) = *T. vulgaris*, n=12; b) = *T. helveticus*, n=693; Mann-Whitney U Test: $z = -2.5765$, $P<0.01$.

differences do occur during the fan display. Male *T. helveticus* have the faster fanning speed (Halliday 1975b, Raxworthy 1989a) and their tail is moved over a horizontal distance of about 1cm, whilst the corresponding distance in *T. vulgaris* is about 1.5cms. Marquenie (1950) and van Gelder (1979) reported differences in the angle of fanning adopted by males, but the results of the two authors for the frequency of fan angles adopted do not entirely agree. Both authors appear to have measured the angle between the body of the male and the female, which is not the most appropriate angle to compare if the fan functions to transfer sensory stimuli because the primary organs for receiving visual, olfactory and

tactile cues are situated on the female's head, not flank. Based on this logic, I compared the angles obtained earlier between the male's body and female's snout for *T. helveticus* with those from 12 unobstructed *T. vulgaris* fan bouts measured in an identical manner (Figure 3.12). Intersnout distances were compared (Figure 3.13) in the same way with a slightly larger sample size (n=20) as differences in intersnout distance between unobstructed and obstructed fan bouts were not significant (Mann-Whitney U test: U= 45.5, P>0.05).

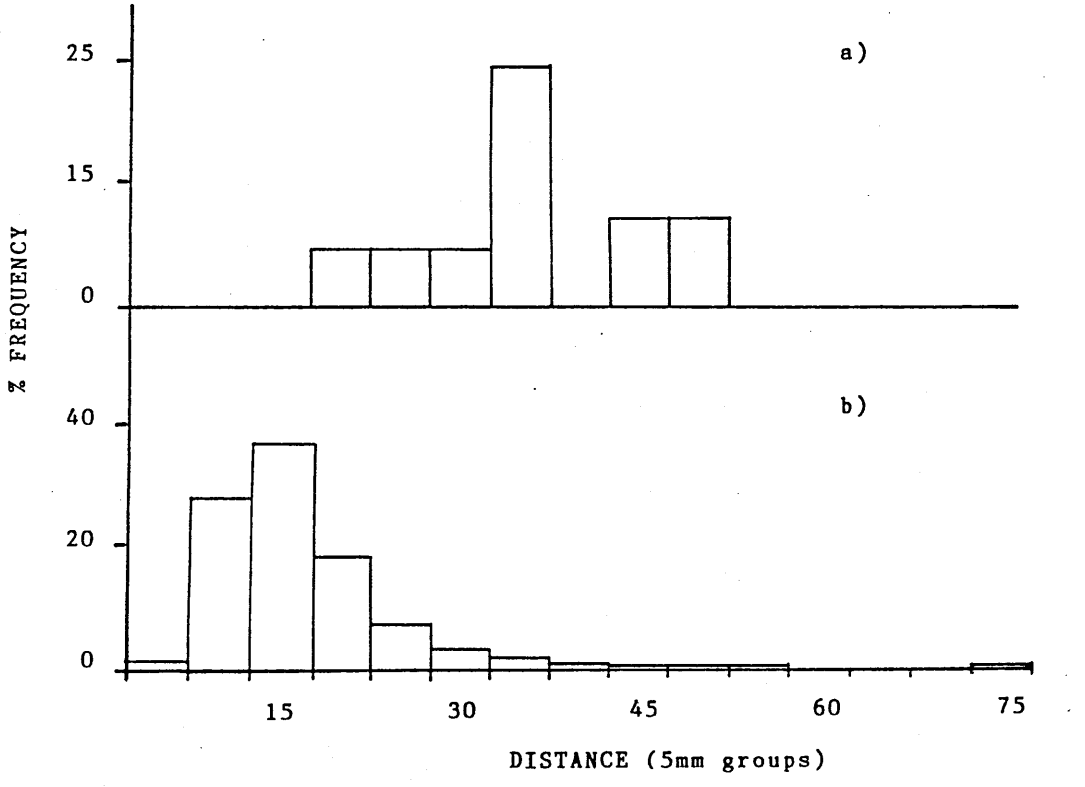


Figure 3.13 Intersnout distance during fan bouts. a) = *T. vulgaris*, n=20; b) = *T. helveticus*, n= 612; Mann-Whitney U Test: z = -6.8644, P<0.0001.

The data, although limited for *T. vulgaris*, nevertheless indicate that male *T. vulgaris* fan at a slightly more obtuse angle and at a greater distance than male *T. helveticus*. However, because there is considerable overlap in the angle of fanning (and some overlap in intersnout distance) it must be

recognised that the stance adopted by the male in isolation, is not entirely reliable as a basis for species recognition by females.

There appear to be only limited differences between homologous display acts, hence it is unlikely that the form of behaviour (when considered alone) is a significant factor contributing to reproductive isolation. However, display may function to increase the efficiency of transfer of other species-specific cues (e.g. morphology and odour) and the minor differences in the form of acts may be a reflection of this potential function. Raxworthy (1989a) has suggested that male courtship display has evolved predominantly to emphasize visual cues in *T. v. vulgaris* and olfactory cues in *T. helveticus*. It is most probable that a female responds to more than one cue, as discrimination is likely to be more accurate if based on a number of sensory modalities (Halliday 1977a).

Differences in the frequency and duration of acts.

A comparison of Halliday's (1972) data for the primary male static and retreat display acts during *T. vulgaris* courtship, with equivalent data obtained here for *T. helveticus* (corrected to account for acts not recognised by Halliday) reveals marked differences between the two species (Figure 3.14). The most common acts performed by male *T. helveticus* are fans, followed by whips, whereas a reversal of the order of these two acts is observed in *T. vulgaris*. A second difference is observed during fan display: male *T. helveticus* fan for longer during uninterrupted bouts than male *T. vulgaris* (Figure 3.15). By repeating these acts more often or for longer, males may be transferring the cues that are amongst the most important for stimulating conspecific females to mate, but which may in turn be either adversive to a heterospecific female, or less effective at persuading her to mate.

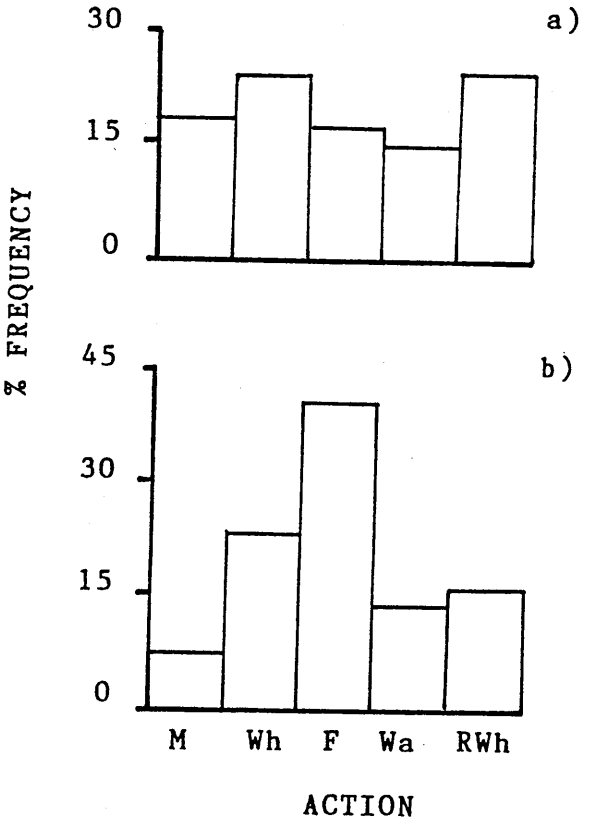


Figure 3.14 Proportion of static and retreat display acts performed. Where a) = *T. vulgaris*, n=2815 (from Halliday 1972); b) = *T. helveticus*, n=4659.

Differences between the transition of acts and pick-up success.

Transitions between some consecutive male acts appear to be influenced by the nature of female behaviour, hence it is necessary to compare the transition in male actions between species during similar conditions of female interruption. Halliday (1975b) investigated first-order transitions between male *T. vulgaris* actions that were uninterrupted, negatively interrupted and positively interrupted and the same analysis has been carried out here for *T. helveticus*. The probability of each category of transition (from M, Wh, -ve/+ve/uninterrupted fan bouts, Wa, R, RWh) within the three female interrupt conditions is shown in Tables 3.11 and 3.12, respectively. As Halliday's data do not distinguish transitions involving wiggles, the transition to the subsequent male action has been substituted where wiggles were performed by male *T. helveticus*, to create a homologous data set for comparison.

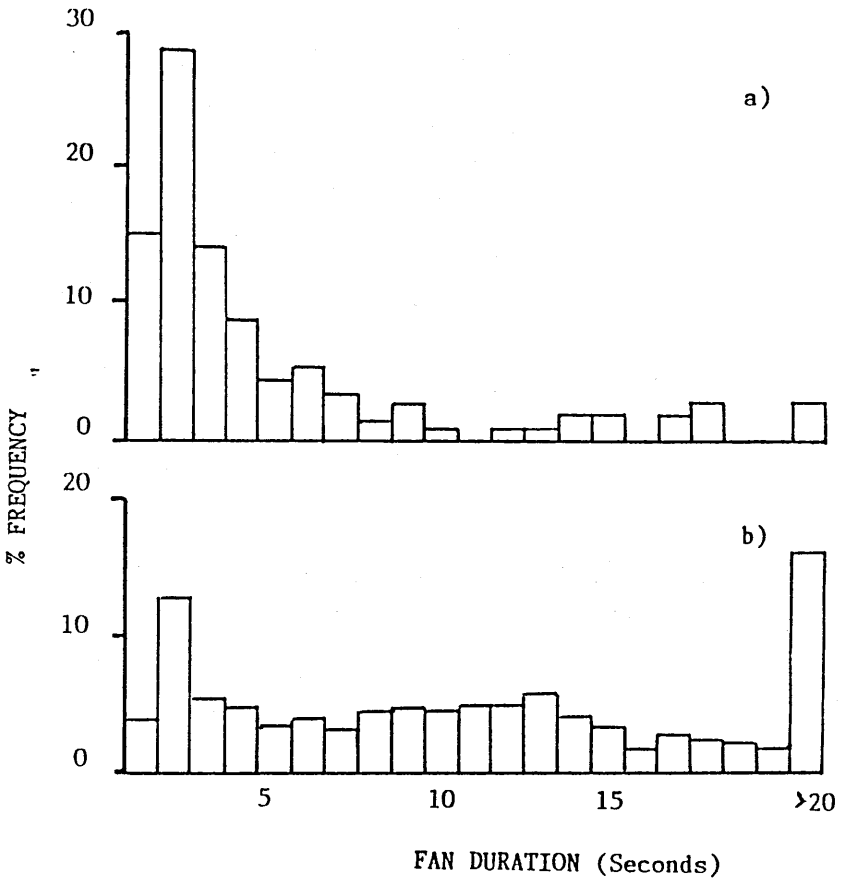


Figure 3.15 Duration of uninterrupted fan bouts. Where a)=*T. v. vulgaris*, n=110 (taken from Halliday 1972); b)=*T. h. helveticus*, n=405.

If the categories of transition with an overall occurrence of less than 1% of the total for each species (i.e. of a frequency of less than 30 for *T. vulgaris* and 12 or less for *T. helveticus*) are excluded by virtue of their low incidence, then only one major difference is observed between the species. This is during uninterrupted transitions from retreat and whip (RWh) behaviour. In *T. helveticus* this act predominantly leads to fanning, whereas in *T. vulgaris* it leads to either fanning or waving (Figure 3.16). It is possible that these differences would influence the outcome of a heterospecific encounter, but it is more likely that other differences in the courtship behaviour of the two species make a greater contribution to sexual isolation than this single factor alone.

	SUCCEEDING ACTS									
PRECEDING ACTS	OR	M	Wh	F	Wa	R	RWh	C	Other	Total
U transitions:										
M	0	6	476	13	0	0	0	0	0	499
Wh	4	17	31	306	45	6	2	11	8	430
F(U)	13	8	8	24	65	1	5	0	1	125
F(-)	20	13	2	2	7	0	0	0	2	46
F(+)	0	1	8	4	14	7	53	7	2	96
Wa	1	13	118	20	69	2	12	0	3	238
R	1	2	8	15	3	0	0	4	8	41
RWh	0	3	8	40	45	4	7	9	1	117
Total	39	63	649	424	248	20	79	31	29	1592
- transitions:										
M	19	0	0	0	0	0	0	0	1	20
Wh	105	19	6	9	6	0	0	0	3	148
F(U)	68	5	1	3	4	0	1	0	2	84
F(-)	47	2	0	0	0	0	0	0	2	51
F(+)	3	0	0	0	0	0	0	0	0	3
Wa	13	2	4	0	6	0	1	0	1	27
R	5	0	0	0	0	0	0	0	2	7
RWh	8	2	2	0	4	0	0	0	0	16
Total	268	30	13	12	20	0	2	0	11	356
+ transitions:										
M	0	1	1	1	0	0	1	0	1	5
Wh	0	1	18	5	9	15	48	7	4	107
F(U)	1	0	14	4	10	3	15	1	3	51
F(-)	0	0	5	0	3	0	3	0	3	14
F(+)	0	0	1	0	0	1	13	2	1	18
Wa	0	0	15	3	8	5	111	7	3	152
R	0	0	3	4	2	38	76	16	3	142
RWh	0	0	6	34	49	64	306	101	8	568
Total	1	2	63	51	81	126	573	134	26	1057

Table 3.11 First-order transitions from male T. v. vulgaris acts. transitions from fan bouts are classified according to whether the bouts from which they arise are uninterrupted (U), negatively interrupted (-), or positively interrupted (+) by the female. Abbreviations: OR = positive orientation acts, M = move-to-the-front, Wh = whip, F = fan, Wa = wave, R = retreat, RWh = retreat and whip, C = creep.

A comparison of Halliday's (1974) data for spermatophore pick-up success in successive *T. vulgaris* sequences (see Table 3.6) shows that the success of consecutive sequences follow opposing trends in the two species (Figure

PRECEDING ACTS	SUCCEEDING ACTS OR	M	Wh	F	Wa	R	RWh	C	Other	Total
U transitions:										
M	0	1	85	2	0	0	3	0	0	91
Wh	8	2	11	254	2	0	0	1	1	279
F(U)	27	1	8	8	28	0	5	1	1	79
F(-)	51	29	4	2	6	0	3	0	2	97
F(+)	12	0	12	2	15	14	82	12	5	154
Wa	8	1	57	14	47	0	16	0	3	146
R	0	2	3	5	1	0	2	0	0	13
RWh	11	0	4	153	0	0	0	0	1	169
Total	117	36	184	440	99	14	111	14	13	1028
- transitions:										
M	0	0	5	0	0	0	0	0	0	5
Wh	6	3	0	7	0	0	0	0	0	16
F(U)	9	3	0	0	3	0	0	0	0	15
F(-)	17	2	0	1	2	0	0	0	1	23
F(+)	0	0	1	0	0	0	1	0	0	2
Wa	6	0	0	0	1	0	0	0	0	7
R	0	0	0	0	0	0	0	0	1	1
RWh	0	0	0	0	0	0	0	0	0	0
Total	38	8	6	8	6	0	1	0	2	69
+ transitions:										
M	0	0	2	0	0	0	0	0	0	2
Wh	0	0	1	2	0	1	1	0	0	5
F(U)	2	0	4	2	9	1	12	0	3	33
F(-)	3	0	5	2	8	0	6	0	0	24
F(+)	3	0	2	0	2	0	17	5	0	29
Wa	2	0	1	0	1	1	4	0	0	9
R	1	0	2	0	1	0	0	0	0	4
RWh	2	0	1	4	1	0	0	0	0	8
Total	13	0	18	10	22	3	40	5	3	114

Table 3.12 First-order transitions from male *T. h. helveticus* display acts. See Table 3.11 for abbreviations.

3.17). It is not known why some sequences are less likely to be successful that others, but these differing trends in pick-up success suggest that the causal relationships between pick-up and non pick-up are not the same for the two species. Direct observational evidence suggests that spermatophores are missed because the female passes to one side of them, but why this should happen more in the earlier *T. vulgaris* sequences, or in later *T. helveticus* sequences, is not apparent.

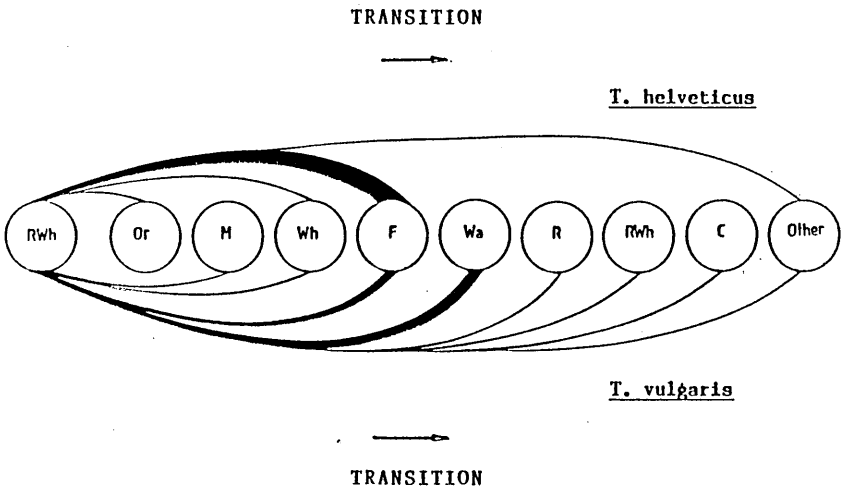


Figure 3.16 Probability of a first-order transition from retreat and whip. Width of transition line is proportional to the probability of the transition. Abbreviations correspond to those listed in Table 3.1.

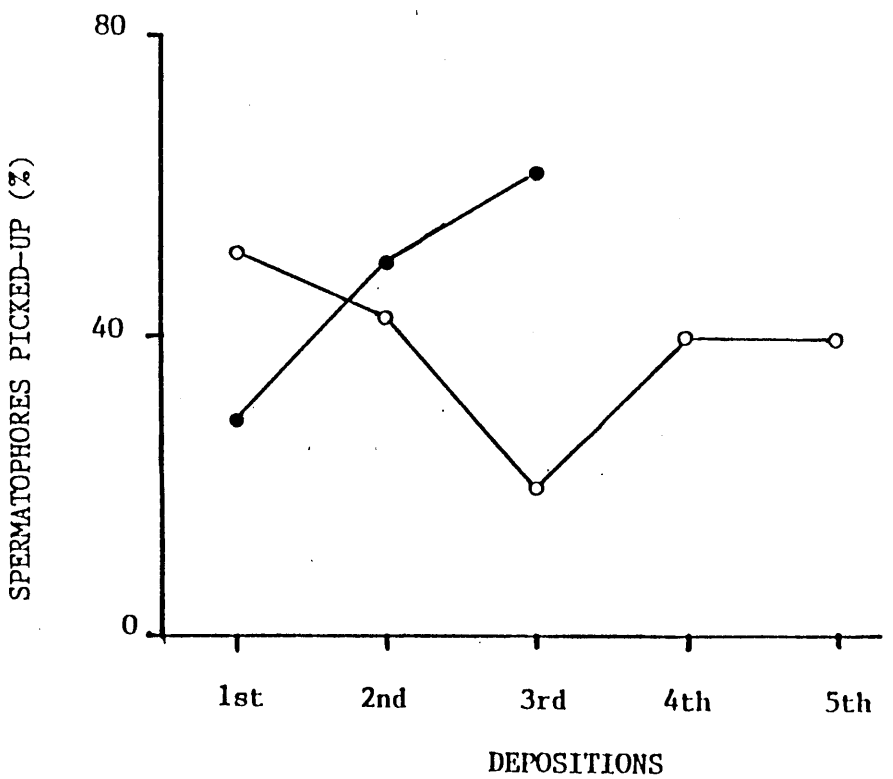


Figure 3.17 Sequence success in *T. vulgaris* and *T. helveticus*. Open circles = *T. vulgaris*, n = 104 (data from Halliday 1974); closed circles = *T. helveticus*, n=104.

Without detailed investigation it is difficult to determine whether these differences would constitue an isolating mechanism. Presumably, if the same trends are found in heterospecific crosses involving a number of sequences, then it is not unlikely that insemination would take place, given two factors which follow similar patterns in both species. First, that a female is as likely to pick-up a heterospecific spermatophore as a conspecific spermatophore if there is contact between it and the female's cloaca (Chapter 1) and secondly, that the probability of an encounter being successful increases with the number of spermatophores deposited (Halliday 1974). However, if the same trends were not found in heterospecific crosses, which may occur as a consequence of a failure in synchronisation (e.g. if the male inappropriately creeps-on, or the female inaccurately follows him, or because of a combination of the two), or as a consequence of physical factors (such as the differing length of the males, or variation in the size of spermatophores), then such anomalies may contribute to reproductive isolation.

MODELS OF SEXUAL ISOLATION - AN OVERVIEW.

The courtship behaviour of the two species differs in a number of aspects, although it is similar in overall plan. Some differences are observed at each of the different stages of courtship, but most commonly they arise in the early stages of an encounter, particularly in relation to the nature and frequency of the fan display. The similarity in form and transition of male acts suggest that qualitative differences in display and order of male acts are not the primary sources of information on which female discrimination is based; those differences in the form of acts which do exist probably function to increase the efficiency of transfer of species-specific sensory cues. The transfer of which is probably enhanced through the differences in relative frequency of male acts.

One model of sexual isolation is that one or both sexes possess a species recognition mechanism in which they fail to recognise heterospecifics as potential mates (*sensu* Paterson 1985). The evidence presented in this and the previous chapter suggests that this mechanism could be based on the morphology or odour of the male, or the morphology or odour of the female, but it would appear unlikely to be based on differences in the form of courtship display. If such a mechanism operates then it would be expected that one or both sexes would not respond to, or would adversely respond to a potential heterospecific mate.

An alternative is that during courtship, a male stimulates a female through a variety of senses and 'persuasion' is achieved by the accumulation of stimuli (Teyssedre & Halliday 1986). As a consequence of the combination of differing display parameters and species-specific sensory cues, heterospecific males may fail to raise female receptivity at a high enough rate to exceed the threshold of positive female behaviour that is the precursor to spermatophore transfer. In this case, the outcome of a heterospecific encounter will, as in conspecific encounters, be heavily influenced by the initial receptivity of the female and the vigour of display of the male, where high initial female receptivity requires relatively less stimulation to exceed a positive behavioural threshold than low initial receptivity and an increase in the vigour of male display provides a higher rate of stimulation. These processes are outlined in the following model (Figure 3.18).

Before proceeding, it is necessary to summarise the transition in female behaviour which normally leads to spermatophore deposition by the male. I have recognised four categories which usually occur over the course of a successful encounter. These are: negative orientation acts, stasis,

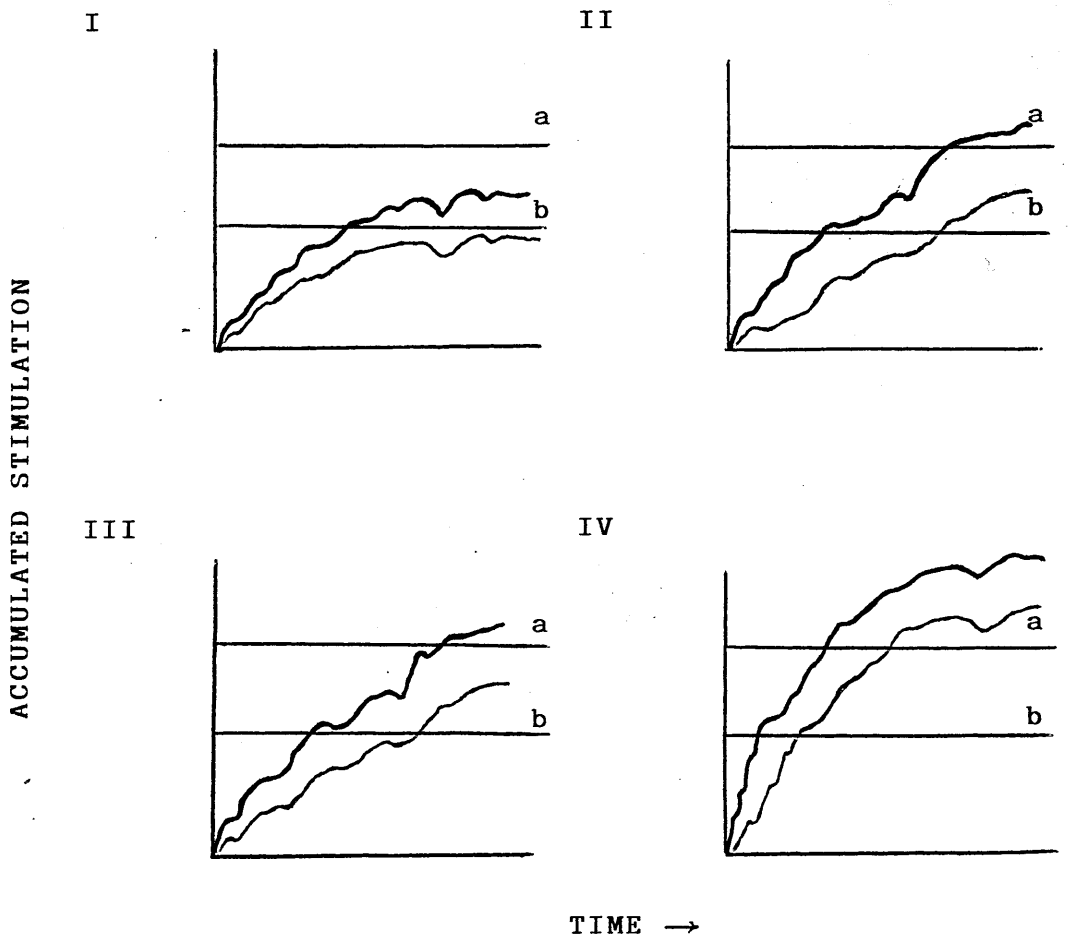


Figure 3.18 Model of sexual isolation in newts. Where: a = threshold of spermatophore transfer behaviour in the female; b = threshold of positive orientation behaviour in the female. Black line = conspecific; red line = heterospecific. See text for explanation.

positive orientation acts and spermatophore transfer behaviour (tail touches). The transition between one of these categories and another may be regarded as the crossing of a behavioural threshold brought about by a change in the sexual motivation of the female. For the purpose of simplicity, only the latter two of these thresholds are shown in the model, but it could equally well apply to all four. The model assumes

that in each scenario the two females are as sexually motivated as each other prior to courtship commencing and that once courtship has commenced, the male displays with equal intensity in both cases.

(I) *Low initial female receptivity and low vigour of male display.*
Here, the male's display provides some stimulation for females of both species, but it accumulates fastest in the conspecific female. The rate of acccumulation is only sufficient to produce positive orientation behaviour (conspecific female) and stasis (hetrospecific female), respectively.

(II) *Low initial female receptivity and vigorous male display.*
The effect of an increase in vigour of male display is to increase the rate of accumulated stimulation in both females when compared to (I). It continues to accumulate fastest in the conspecific female and is sufficient to elicit spermatophore transfer behaviour. However, only positive orientation behaviour is achieved in heterospecific females.

(III) *High initial female receptivity and low vigour of male display.*
The vigour of male display is the same as that in (I), but as the females initial receptivity is higher, the rate of accumulated stimulation is increased and is now sufficient to bring about a behavioural transition. Here, the conspecific female moves quickly from positive orientation behaviour to spermatophore transfer behaviour, whilst in the heterospecific female only positive orientation behaviour is elicited.

(IV) *High initial female receptivity and vigorous male display.*
In both females in this scenario receptivity is higher here than in the corresponding female in (I) and (II). Combined with a male display more

vigourous than that in (I) and (III), an increased rate of accumulated stimulation is produced which although faster in the conspecific female, is sufficiently greats to produce spermatophore transfer behaviour in both cases.

When applied to natural courtship, this model is probably an over-simplification. Nevertheless, it provides an explanation of the processes which may bring about sexual isolation, despite courtship by a heterospecific male being initiated. It also provides an explanation of the process which would allow hybridisation to take place: in (IV), an initially highly receptive female is courted by a vigorously displaying heterospecific male. Females which have been previously courted (but not inseminated) by a conspecific male are likely to have a relatively high initial receptivity if subsequently engaged in courtship by a hetero-specific male. It was under such conditions that Halliday (1977a) reported that interspecific matings took place between *T. vulgaris* and *T. helveticus*; such a condition may also arise as a consequence of sexual interference in a conspecific courtship encounter by a heterospecific male.

The behaviour of potential mates during interspecific courtship encounters is investigated in the following chapter.

CHAPTER 4

SPECIES DISCRIMINATION

Tinbergen (1953) proposed that one of the functions of courtship behaviour was to maintain reproductive isolation, ensuring that only individuals of the same species mate with one another. The previous chapters focused on interspecific differences in the morphology and sexual behaviour of *T. vulgaris* and *T. helveticus* which may serve as isolating mechanisms between the two species. There are no detailed accounts specifically investigating pre-mating mechanisms of reproductive isolation in *T. vulgaris* and *T. helveticus* in the literature, but preliminary experiments by Halliday (1977a) to investigate the possible role of courtship indicated that selective responsiveness by females to conspecific and heterospecific male display is responsible for pre-mating reproductive isolation. He found that some female *T. helveticus* responded to *T. vulgaris* courtship with atypical, jerky movements and that spermatophores were picked up, but positive behaviour was only evoked in females after they had been courted by conspecific males. When there was no prior conspecific courtship, females were unco-operative with heterospecific males. In contrast, males displayed to both conspecific and heterospecific females with equal intensity, suggesting that they lacked the ability to discriminate, at least in captivity.

The main aim of this chapter is to provide the first detailed quantitative investigation of the response of male and female *T. vulgaris* and *T. helveticus* during conspecific and heterospecfic encounters and of the role of courtship as a possible ethological isolating mechanism.

Receptive newts of either sex respond to their partner by performing positive acts (Nelson 1959, 1960; Halliday 1977a) such as orientation and in the case of male newts, display. Positive responses are used as criteria for determining preference and hence, discrimination in this study. In the first series of experiments, the animal responsible for terminating the last encounter of a trial has also been noted, as rejection of a mate constitutes a strong component of selectiveness that may also be used to assess discrimination ability. Halliday's (1977a) limited data regarding heterospecific encounters in newts suggests that discrimination wanes in highly motivated females. It is therefore possible that selectiveness in newts may be related to the motivational state of the partners. In a second series of experiments, the response of male newts to conspecific and heterospecific females artificially manipulated to mimic positive or negative motivational states, is also examined.

EXPERIMENT ONE - MATING TRIALS.

Method

Aquatic newts captured at the ponds listed in Table 2.1 were used in this and the following experiment. They were maintained under the same general conditions reported in Chapter 1. Trials were carried out in April and May 1982 and March to June 1983 using animals chosen at random.

Prior to the trials a short test was carried out on male *T. vulgaris* and *T. helveticus* to determine whether they were sexually responsive. A conspecific female was anaesthetised in a solution of m-aminobenzoate (MS222) and placed in a straitjacket harness (a slit plastic tube attached to a hand-held rod - see Halliday 1975b). The harnessed female was lowered onto the substrate and brought slowly toward the male from the opposite side of the tank. Once their snouts were 1cm apart the male was

allowed 30 seconds to court the female. If he performed one or more courtship display acts he was judged to be sexually responsive and suitable for use in the following experiment. Male *T. helveticus* exhibited an extreme aversion to straitjacketed conspecific females (possibly because of their unusual appearance) and could not be used in the following experiment.

Once a minimum duration of 10 minutes had elapsed after the test for receptivity and after the male had ascended for air, an unrestrained female newt was placed into the aquarium with the male. The interactions of the animals were recorded on video. If at the end of a ten minute period there had been no encounters (defined as a meeting of the partners within one body length of the snout), the trial was abandoned and repeated with a different pair of newts. However, if the pair were engaged in an active encounter (e.g. Oh+, Sn, Wh, etc.) at the end of this period, the trial was extended until the end of the encounter. Twenty trials with male *T. vulgaris* paired with conspecific females and 20 trials with male *T. vulgaris* paired with heterospecific females were obtained in which there was at least one encounter. Behavioural acts were transcribed off the video recordings onto paper to the nearest 0.01s and two-tailed Mann-Whitney U Tests were carried out on the interval between the female entering the water and the first encounter, the number of sniffing bouts performed by the male, the total number of positive orientation acts (e.g. Oh+, Or) performed by each partner and the total number of static and retreat display acts (e.g. M, RWh) and creeps performed by the male in trials in which courtship display occurred, to determine whether significant differences in response were performed toward females of the two species. In addition a note was made of the sex of the newt responsible for initiating the first encounter and also ending the last

encounter of a trial and also the number of spermatophores transferred. As the majority of frequency distributions of acts did not appear to be normally distributed, non-parametric descriptive statistics (i.e. median and 95% confidence limits) have been used.

FEMALE PARTNER	INITIATOR Female	Male	Binomial Test
T. vulgaris	9½	10½	P>0.05
T. helveticus	5½	14½	P>0.05

Table 4.1 Partner responsible for initiating the first encounter with a *T. v. vulgaris* male.

Results

An encounter was usually initiated when one of the partners walked toward the other as they wandered around by the walls of the aquarium. In both conspecific and heterospecific trials, either partner was equally likely to initiate the first encounter (Table 4.1). Although there was a tendency for conspecific encounters to begin with shorter latency (Table 4.2) than heterospecific encounters, these intervals did not differ significantly.

Female Partner	Latency (secs) median	95% cl	Mann-Whitney U Test n	U	P
T. vulgaris	33.4	12.3-81.0	20	140	>0.05
T. helveticus	86.3	46.6-186.0	20		

Table 4.2 Latency to first encounter.

When the response of male newts toward conspecific and heterospecifc females were compared for all trials, no significant differences were found in the number of positive orientation acts, or in the number of sniffing

bouts (Table 4.3). Female newts rarely sniffed their partner; male *T. vulgaris* sniffed conspecific and heterospecific females in 70% and 90% of trials, respectively.

MALE RESPONSE	NUMBER OF RESPONSES TO FEMALE *T. vulgaris* total	m	95% cl	*T. helveticus* total	m	95% cl	M-W U Test* U	P
Positive orientation acts	357	13	7-22	205	17.5	6-10	145.5	>0.05
Sniffing	82	2	0-5	73	3	2-5	168.0	>0.05

Table 4.3 Number of positive orientation acts and sniffing bouts performed by male *T. vulgaris* to unrestrained conspecific and heterospecific females. * = number of pairs: *T. vulgaris*, n=20; *T. helveticus*, n=20.

A comparison of the number of positive orientation acts performed by female *T. vulgaris* and female *T. helveticus* toward courting and non-courting male *T. vulgaris* (Table 4.4) revealed significant differences, with females of both species responding positively most frequently when courted. However, female *T. helveticus* responded with fewer positive orientation acts than female *T. vulgaris* during courtship encounters.

FEMALE PARTNER	ENCOUNTERS courtship med	range	n	non-courtship med	range	n	Mann-Whitney U Test U	P
T. vulgaris	6	0-3	11	1	0-7	9	19.5	<0.05
T. helveticus	2	1-4	7	0	0-2	13	12.0	<0.01
Mann-Whitney U Test	U	15.5			37.0			
	P	<0.05			>0.05			

Table 4.4 Number of positive orientation acts performed by female newts toward courting and non-courting male *T. v. vulgaris*.

Male *T. vulgaris* performed one or more display acts in eleven (55%) conspecific trials and seven (35%) heterospecific trials. In nine of the former and five of the latter the female partner terminated the courtship encounter. Waves (37.4%) and whips (29.1%) were the two most common static display acts performed by the male newts toward their conspecific partners, with RWh's (9.6%) being the predominant retreat display act performed (Table 4.5). In contrast, waves (53.2%) and fan bouts (15.4%) were the most common static display acts and RWa's were the only retreat display act performed to female *T. helveticus*. Male *T. vulgaris* court female *T. vulgaris* and *T. helveticus* with equal vigour (Table 4.5).

		FEMALE	
DISPLAY ACT		*T. vulgaris*	*T. helveticus*
Static display			
M		8.3%	12.2%
Wa		37.4%	53.2%
F		14.8%	15.4%
Wh		29.1%	14.7%
Retreat display			
RWa		-	4.5%
RF		0.9%	-
RWh		9.6%	-
Total number of acts		230	156
Median (20 trials)		14	10
95% conf. limits		9-28	5-32
Mann-Whitney	n=	11	7
U Test	U=	150.5	
	P=	>0.05	

Table 4.5 Frequency of courtship acts performed by male *T. v. vulgaris* to conspecific and heterospecific female newts.

Of the 18 trials in which courtship behaviour was observed, only one, between conspecific partners, resulted in spermatophore transfer. The low incidence of spermatphore transfer in conspecific encounters suggests that the majority of female newts which were courted were not sexually

motivated. Their low receptivity may be partly due to insufficient time to recover from the effects of transfer into the aquarium.

The duration of trials ranged between 600s and 865s for conspecific partners and 600s and 1338s for heterospecific partners. During the longest heterospecific trial the male maintained a prolonged period of courtship display and performed 52.8% more static display acts towards his partner than was required by the only female *T. vulgaris* to progress to the spermatophore transfer phase (Table 4.6). Despite sustained courtship, the female *T. helveticus* remained unreceptive and the courtship sequence did not progress beyond static display.

	NUMBER OF DISPLAY ACTS PERFORMED					
	static display				retreat display	spermatophores deposited
FEMALE PARTNER	M	Wa	F	Wh	RWh	S↓
T. vulgaris (Con)	3	42	6	19	7	1
T. helveticus (Het)	15	51	21	20	0	0

Table 4.6 The number of courtship display acts performed during the most vigorous trials with conspecific and heterospecific females.

The partner responsible for terminating the last encounter of a trial is shown in Table 4.7. By turning and either walking or swimming away, female *T. vulgaris* ended the majority of final encounters. This again suggests that female *T. vulgaris* were unreceptive to male newts. The tendency shown by male newts to terminate the last heterospecific encounter was not significant, which indicates that both male *T. vulgaris* and female *T. helveticus* were equally likely to end a heterospecific encounter.

TRIAL	PARTNER ENDING TRIAL Female	Male	BINOMIAL TEST
Conspecific	17½	2½	P<0.01
Heterospecific	13½	6½	P>0.05

Table 4.7 Partner responsible for ending the last encounter of a trial.

Discussion

In both the conspecific and heterospecific trials, both sexes were equally likely to initiate the first encounter. This, combined with the data indicating that there were also no differences in the latency to first encounter in both series of trials suggests two things. First, that encounters were initiated by chance, as one or both partners wandered around the aquarium and secondly, that there is no evidence to suggest that either the male partners actively sought either conspecifc or heterospecifc females, or that female partners of either species sought male *T. vulgaris*, prior to an encounter beginning. The confinement of an aquarium probably improved the possibility of two potential partners meeting when compared to a field situation where there is a large expanse of substrate and because of this, assumptions that male and female newts are not attracted to potential partners from a distance (possibly by visual or olfactory cues), or that discrimination from a distance does not occur, should be made with caution. Indeed, in low density populations particularly, selection pressures may favour newts which have evolved a mechanism of attraction which improves the probability of conspecific partners meeting and mating.

Sniffing bouts were frequently performed by male *T. vulgaris* during both conspecific and heterospecific encounters, but no selective sniffing behaviour was found toward female *T. vulgaris*, or female *T. helveticus*. It

may be that olfactory cues relating to the female's species, reproductive condition, or motivational state are obtained by the male during sniffing bouts and that olfactory cues may attract, stimulate, or repel the male, but no evidence is provided by this experiment to support any of these possibilities.

The results indicated that under laboratory conditions, female *T. vulgaris* and *T. helveticus* did not show selective behaviour to male *T. vulgaris* in the absence of courtship display. Where there was courtship, females of both species performed more positive acts toward their partner than females which were not courted, although this response was weaker in female *T. helveticus*. Thus, under laboratory conditions, female *T. helveticus* will respond to the display of heterospecifc males, although as found here, not strongly enough to elicit spermatophore transfer behaviour in the male. This suggests that interspecific encounters fail as a consequence of inadequate response to heterospecific courtship, rather than by recognition and avoidance of heterospecific males based on sensory cues received prior to courtship commencing. It provides evidence to support the hypothesis that sexual isolation occurs because heterospecific males do not raise female receptivity at a high enough rate.

Only one of the conspecific courtship trials resulted in spermatophore deposition, which suggests that the majority of female subjects were probably not sexually motivated and could not be persuaded to be so, despite the increased level of positive responses elicited by male courtship. This is supported by the findings that female *T. vugaris* were responsible for terminating final encounters on a greater number of occasions than conspecific males. Although the results of the latter heterospecifc analysis contrast with the results for conspecific partners

(the number terminated by male *T vulgaris* did not differ significantly from that terminated by female *T. helveticus)*, they do not necessarily detract from the earlier conclusion, as it is possible that the male newts which ended the heterospecific encounters did so because they were not sufficiently motivated to continue an encounter with a heterospecific female that was unreceptive. As insufficient time to recover from the effects of transfer may have been a contributing factor to the apparent lack of sexual motivation in females, one must assume that future experiments would benefit from the incorporation of a recovery phase prior to the start of the trial.

EXPERIMENT TWO - THE IMPORTANCE OF FEMALE MOTIVATION.

In the previous experiment male *T. vulgaris* did not show selective behaviour toward females of either species in both courtship and non-courtship trials. Male *T. vulgaris* may however, discriminate between conspecific and heterospecific females that are sexually motivated, as Liley (1966) reported that male discrimination between species of Guppies depended on selective responsiveness of females, which respond positively only to conspecific males. To test this, the response of unrestrained male *T. vulgaris* paired with both a female *T. vulgaris* and *T. helveticus* artificially manipulated to mimic positive or negative motivational states were investigated.

Method

Two series of trials were carried out. In the first, or 'receptive' female trials, 30 male *T. vulgaris* were allowed ten minutes to recover from the effects of transfer into the aquarium before being presented consecutively with an anaesthetised straitjacketed female *T. vulgaris* and *T. helveticus.* After the male had ascended for air and returned to the substrate a

harnessed female was brought slowly toward the male until the partners were positioned 1cm. apart with snouts' facing. The female was then manipulated to mimic periods of stasis followed by shorter periods of positive orientation acts concordant with sexually responsive behaviour. Three periods of static/positive 'behaviour', each of which consisted of 30 seconds of stasis followed by 10 seconds of positive advance, were performed in succession. One exception to this was when a male ascended for air and immediately returned to his partner. In such cases the remainder of the phase was completed on his return. If the male went away from the female under any other circumstances, a 60 second interval was allowed before the female was brought back into position for the next static/positive period. If during the trial the male initiated spermatophore transfer by creeping, the normal tail-touch reponse of a receptive female was withheld. At the end of the three static/positive periods the male was allowed a ten minute interval and to ascend for air before the second female was presented in the same manner. The order of species presentation was alternated to control for position effects.

In the second series of 'unreceptive' female trials, pairs of harnessed conspecific and heterospecific females were manipulated to mimic the periods of stasis and negative orientation acts concordant with unreceptive female behaviour. Three consecutive periods of 30 seconds of static and 10 seconds of negative behaviour were again performed. If the male either went away from the female at any stage (except when he returned after ascending for air), or failed to follow the female after a negative phase, a 60 second interval was allowed before the female was brought back into position for the next static/negative period. In all other respects the first and second series of trials were carried out in a similar fashion.

The sequences of events for both series were transcribed onto paper to the nearest second using a stopclock. The total time spent by the male facing the female within one half a body length and the frequency of positive orientation acts, sniffing bouts and display acts performed were noted. The response of males to conspecific and heterospecific females were compared using two-tailed Wilcoxon Matched-Pairs Tests corrected for ties; similar tests were carried out for all positive acts on the first and second females presented to determine whether order of presentation affects male response.

Results

The male subjects did not show any consistent adverse or abnormal behaviour toward harnessed females of either species. Males remained with conspecific females for longer duration than heterospecific females in the static/positive trials, but no significant preference emerged in static/negative trials (Table 4.8).

		PROXIMITY DURATION (seconds)			
		static/positive		static/negative	
STATISTICS		*T. vul*	*T. hel*	*T. vul*	*T. hel*
Median		86.5	72.0	62.5	64.5
95% Conf. limits		63-105	54-83	54-84	49-84
Wilcoxon	n*	29		30	
Matched	T	83		227.5	
Pairs Test	P	<0.01		>0.05	

Table 4.8 Duration spent by male *T. vulgaris* in proximity to 'receptive' and 'unreceptive' conspecific and heterospecific females. * = corrected for ties.

Positive orientation or display acts were performed by males in 93.4% of static/positive trials and 86.7% of static/negative trials. The two most common positive orientation responses were head turn and positive advance,

NUMBER OF POSITIVE ACTS	TRIALS			
	static/positive		static/negative	
	T. v	*T. h*	*T. v*	*T. h*
Orientation				
Air↓	6	8	3	1
Oh+	23	23	27	25
Sw+	2	1	3	4
Or	2	2	7	4
+Adv	23	11	22	18
Fo	0	0	0	1
Display				
M	1	0	1	4
Wa	72	40	44	42
F	7	3	12	16
Wh	39	13	26	17
RWh	10	3	0	0
C	2	0	0	0
Total	187	103	145	132
Median	4	2.5	3	2.5
95% conf. limits	2-7	2-3	1-5	1-4
Wilcoxon Matched Pairs Test	n*	27	21	
	T	93.5	72.0	
	P	<0.05	>0.05	

Table 4.9 Number of positive acts performed by male *T. vulgaris* to 'receptive' and 'unreceptive' conspecific and heterospecific females.
* = corrected for ties.

whilst waves and whips were the most frequently performed display acts in each series. Of the 19 males which courted, nine courted females of both species, eight courted conspecific females only and two courted heterospecific females only. Static display acts were performed in trials involving both 'receptive' and 'unreceptive' females. As expected, no retreat display or spermatophore transfer acts were performed towards females exhibiting 'unreceptive' behaviour (Table 4.9). However, spermatophore transfer was initiated by one male only, when paired with a conspecific female in a static/positive trial. This male creeped after just five seconds of courtship display, which suggests that he was probably sexually highly motivated. Although he was also very responsive

to the female *T. helveticus* subsequently presented to him and performed more display acts to her than to his conspecific partner (21 acts compared to 13), he did not initiate spermatophore transfer in the heterospecific trial.

When the number of all positive acts (orientation and display) performed by male *T. vulgaris* toward conspecific and heterospecific females were compared (Table 4.9), a preference emerged for 'receptive' conspecific females over 'receptive' heterospecific females, but none was found for 'unreceptive' females. The preference observed was largely due to a tendency to perform more display acts to conspecific females than heterospecifc females (Wilcoxon Matched-Pairs Test: n=19, T=47, $P<0.10$); the results suggest that male *T. vulgaris* court 'unreceptive' female *T. vulgaris* and *T. helveticus* with equal vigour. No significant differences were found in the number of all positive acts performed towards first and second order females in either series (Wilcoxon Matched-Pairs Tests: static/positive tests - n=27, T=143, $P>0.05$; static/negative tests - n=21, T=60, $P>0.05$).

The statistical tests (Table 4.10) indicate that male *T. vulgaris* do not selectively sniff either 'receptive', or 'unreceptive' conspecific females over heterospecific females and nor do they selectively sniff 'receptive' female partners over 'unreceptive' female partners of the same species.

Discussion

The results suggest that during an encounter male *T. vulgaris* may show preferential selectivity, dependant on a receptive motivational state in the female. However, as a number of male *T. vulgaris* vigorously courted female *T. helveticus* during both experiments, I must conclude that the

ability of male *T. vulgaris* to discriminate against heterospecifc females is weak, at least in the laboratory.

		PARTNER						Wilcoxon Matched Pairs Test		
		T. vulgaris			*T. helveticus*					
SERIES		m	95% cl	Σ	m	95% cl	Σ	n*	T	P
static/+ve		1	0-2	37	1	1-2	37	20	101	>0.05
static/-ve		0	0-1	29	0	0-1	22	18	72.5	>0.05
Mann Whitney U Test	n		30,30			30,30				
	U		368.5			400				
	z		1.6902			1.0369				
	P		>0.05			>0.05				

Table 4.10 Median number of sniffing bouts performed by male *T. vulgaris* to 'receptive' and 'unreceptive' conspecific and heterospecific females. * corrected for ties.

It is possible that selectiveness in males is based predominantly on strength of female response rather than on whether a female is simply receptive or unreceptive. Two things suggest that male discrimination also involves other factors. First, in experiment one, conspecific females responded with more positive actions that heterospecific females, yet the strength of male response was equal. This is not what would be predicted if female response was the only important factor. Secondly, the manipulated response of the two species of female in both the static/positive and static/negative trials of experiment two were equivalent, yet the strength of male response was unequal. Again, this would not be predicted if strength of female response alone was responsible for male selectiveness. I propose that strength of female response is probably an important factor in discrimination by male *T. vulgaris*, but it is not the only one. Contributing factors may relate to the differences in external morphology observed between female *T. vulgaris* and *T. helveticus*, or in differences perceived by other sensory modalities

such as olfaction. It is possible that even a weak degree of male selectivity (manifest as shorter bouts of continuous display, or reduced display rate) may prevent accrual of the effects of male display during heterospecific encounters.

Liley (1966) found that male discrimination betwen species of female guppies was dependant on positive reinforcement from conspecific females, but that it was only after experience of heterospecific females failing to respond that male fishes learnt to restrict their courtship to conspecific females. In this experiment male newts were obtained from populations in which *T. vulgaris* and *T. helveticus* were syntopic and allotopic. Exact details of the experience of individual newts is unavailable but it is possible that some of the male subjects from syntopic populations had prior experience of the response of both conspecifc and hetrospecifc females to courtship, whilst the others from allotopic populations only had experience of conspecific female response. Thus, individual experience may have modified the response of males in experiments one and two.

The degree of selectivity observed appears to be stronger in female *T. helveticus* than male *T. vulgaris*. However, this is not unusual as asymmetric selectiveness has been observed in other species. Bateman (1948) found that female *Drosophila melanogaster* are responsible for ending conspecific courtship, however, male *D. melanogaster* and *D. simulans* (a sibling species), are predominantely responsible for ethological isolation (von Schichler and Dow 1977). In contrast to male *D. mojavensis* which exhibit precourtship discrimination, male *D. arizonensis* continue to court heterospecific females, even though they do not obtain the acceptance gesture required for copulation from them (Markow 1981). Salmon (1965) found that male Fiddler Crabs (*Uca pugilator* and *U. tangeri*) fail to

discriminate between females of different species and he concluded that females must be the sex responsible for reproductive isolation. Wilz (1970) observed that male Three and Ten-spined Sticklebacks were equally willing to court females of either species, but found that females preferred conspecific males.

There are as yet, no laboratory studies in the literature to investigate the degree of selectivity operated by female *T. vulgaris* and male *T. helveticus* to prevent mismatings. When {male *T. vulgaris* x female *T. helveticus*} and {male *T. helveticus* x female *T. vulgaris*} are considered as two groups of potential heterospecific mates, the sex/species that produces the fewest number of mature gametes would gain the most from early evolution of an accurate discriminating ability, assuming that such an ability was not already effective in an heterospecific partner of higher fecundity when the two species first became sympatric. It is generally thought that there is a disparity in the reproductive investment of male and female newts (Halliday 1977), with males being potentially capable of producing a greater number of gametes during a breeding season than females. This suggests that female newts should be the more discriminating sex (which is supported by the results of experiment one) and hence, that female *T. vulgaris* would show stronger selectvity than male *T. helveticus*. These predictions are made with some caution, as experiments comparing the full reproductive potential of male and female *T. vulgaris* and *T. helveticus* are lacking.

Halliday's (1977a) limited data regarding heterospecific encounters indicates that discriminating ability wanes in highly motivated females. If highly motivated females in the natural environment become unselective, it would be advantageous for male newts to possess some degree of

selectivity to avoid wasting a proportion of their sperm supply, which is limited (Lofts 1984, Verrell *et al.* 1986). However, retention of sperm is not the only benefit that might accrue from male discrimination. Courtship necessitates vigorous and sustained movement in male newts and reducing intensity or duration of courtship display to heterospecific females may decrease both wasted expenditure of energy and possible exposure to predators. Furthermore, the courtship phases in the field are predominantly crepuscular, with the peak occuring around dusk (Griffiths 1985, Verrell & McCabe 1988, pers. obs. - see Chapter 1). A male would not be wasting as much of the limited optimum time available to him in which he could otherwise be courting a conspecific female if he excercised some degree of selectiveness, even if this was only evoked in the later stages of courtship. I am unable to predict whether male *T. vulgaris* will be more or less discriminating than male *T. helveticus* because little is known about the quantity of sperm available to male newts and there is currently no evidence to indicate which species is capable of fertilizing more eggs during their reproductive lifetime.

The prevalence of sniffing bouts performed by males in these experiments again suggests that olfactory cues are important during an encounter, although lack of selectiveness shown to conspecific and heterospecific females of both motivational states indicates that the number of sniffing bouts performed is an inadequate criterion for assessing the relevance of this act to male *T. vulgaris*. Without additional evidence, sniffing cannot be accepted as an indicator of preference, either in terms of species discrimination, or when comparing response of females of different motivational states.

SPECIES DISCRIMINATION - AN OVERVIEW.

The evidence in this chapter suggests that a complex interactive process takes place between the sexes once an interspecific encounter is initiated, which contributes to sexual isolation. This process is briefly outlined in the following synthesis:-

Once heterospecific courtship commences, the rate of accumulation of stimulation produced by the male's display is inadequate to raise female motivation sufficiently to enable spermatophore transfer behaviour responses to be elicited. As the encounter continues, a negative feedback system becomes operative, in which the lack of responsiveness in the female, combined with other factors such as limited oxygen supply, leads to a reduction in vigour of the male's display, which in turn reduces the rate of stimulation he provides for the female. This process continues and leads to a breakdown of the encounter.

The importance of species-specific cues (which may influence this process) is investigated in the following chapter.

CHAPTER 5

THE IMPORTANCE OF VISUAL, OLFACTORY AND TACTILE CUES.

The importance of differing morphology, water-borne odours and the tactile and visual cues of movement produced during the fan display are each investigated in the following three sections.

VISUAL CUES.

Visual cues have often been identified as potential sources of species-specific information. Examples which are thought to play a role in sexual isolation include wing posture in the *Drosophila obscura* species group (Brown 1965), light-flashes in fire-flies (Schaller & Schwalb 1961), duration of waving display in *Uca* crabs (Salmon *et al.* 1979), head-bobbing in lizards of the *Sceloporus torquatus* group (Hunsaker 1962), dewlap colour in *Anolis* lizards (Williams & Rand 1977), wing colouration in *Calopteryx* damselfies (Waage 1975), eye contrast pattern and wing colouration in arctic gulls (Smith 1966), plumage differences in both grosbeaks (Kroodsma 1974) and buntings (Emlen *et al.* 1975) and head and body shape in Darwin's finches (Ratcliffe & Grant 1983).

There are many differences in the external morphology of male and female *T. vulgaris* and *T. helveticus* (Chapter 2) and the variations in colour, contrast, patterning, size and shape may individually, or in combination, provide visual cues that both male and female newts may recognise during or prior to courtship. Differences in the display actions of courting males of the two species (Chapter 3) may also be important by providing the female with additional visual cues of movement which may facilitate any mating process. A number of components of visual stimuli may

contribute to species recognition (Madison 1977) and if either males or females show some selectivity in favour of conspecifics, when based on visual cues, then this may contribute to sexual isolation.

Associations between the visual system, physiology and reproductive activity in *T. vulgaris* and *T. helveticus* are not precicely known. I obtained preliminary evidence that visual cues are important during conspecific courtship from observations of a male *T. helveticus* with one abnormal eye courting a female of the same species in the laboratory. When the female moved onto his 'blind side' he stopped displaying to her, but resumed when she moved onto the side of his normal eye. The same phenomenon has been observed by Halliday (pers. comm.) in *T. vulgaris*. Malacarne and Giacoma (1980) showed that courtship behaviour toward conspecific females was reduced but not lost entirely in male *T. (cristatus) carnifex* with permanent lesions to the pre-optic area. The testes of these lesioned animals showed regressive changes but Malacarne and Giacoma concluded that further work was necessary to distinguish between the neural, hormonal and neuroendocrine functions of this region.

Little work has been published regarding the visual system of *T. helveticus*, but a detailed description of *T. vulgaris, T. cristatus* and *T. alpestris* has been provided by Grusser-Cornhels and Himstedt (1976). Only 13% of the receptors on the retina of *T. vulgaris* are rods (Moller 1951) and the predominance of cones indicates that newts of this genus have excellent colour vision (Himstedt 1973), which has been supported by some experimental evidence. Grusser-Cornhels and Himstedt found that terrestrial *T. vulgaris* show the greatest number of head turns in response to red wavelengths, decreasing in number in order to yellow, blue and finally green wavelengths. In springtime, the spectral sensitivity of

newts shifts to yellow and red colours (Himstedt 1973) and newts orientate well to the colours that predominate during the breeding phase; in male *T. alpestris*, the ability to distinguish colours is used in the recognition of conspecific females (Himstedt 1979). Using female models, Himstedt (1979) found that the response of male *T. alpestris* was predominantly the result of stimuli relating to colour pattern and the position of colour on the appropriate parts of the female's body and not of patterns of colour contrast, although males responded to a pattern of colours that contained some contrast between the belly and dorsum.

The visual stimuli produced by crude female models are sufficient to induce courtship in male *T. vulgaris* (e.g. Grant 1966, Halliday 1975b), but my observations indicate that male *T. helveticus* generally show extreme aversion to both models and anaesthetised harnessed conspecific females (Chapter 4). Given the limitations to experimentation of such sources of visual stimulation, I used a modificaton of Verrell's successful experiments used with both the Red-Spotted newt, *Notophthalmus viridescens* (Verrell 1985) and *T. vulgaris* (Verrell 1986b), recording proximity and behaviour of subjects to animals restrained under paired watertight glass containers as the initial basis for determining visual selectivity and hence, discriminaton. Live, un-anaesthetised newts provide additional visual stimuli over models by virtue of their general activity; this may be important to a potential mate, as newts have been shown to respond to small movements (Cooke & Fulford 1974, Grusser-Cornhels & Himstedt 1976).

Method

Experiments were carried out from 6.00 - 10.30 hrs and 16.00 - 21.30 hrs GMT during May and June 1982 and from March to May 1983. The apparatus (Figure 5.1) consisted of a glass aquarium (61x30x30 cms) surrounded by

black paper to exclude visual stimuli, filled to a depth of 15cms with aged tap-water at a temperature of 19±3°C. Low intensity illumination was provided by dimmed spot-lights or angle poise lamps. The floor of the tank was covered by a sand and fine gravel substrate, and metal wires, shaped to form three concentric rings 4.5cms apart, were laid on each side of the aquarium equidistant from the mid-line. An inverted 250ml glass evaporating dish in which an air-pocket had been trapped was placed in each of the two inner rings and these were pushed into the substrate to a depth of 1cm to form the system in which stimulus animals were restrained; Verrell (1985) tested a similar system with dyes and concluded that under these conditions his containers were waterproof and presumably, odourproof.

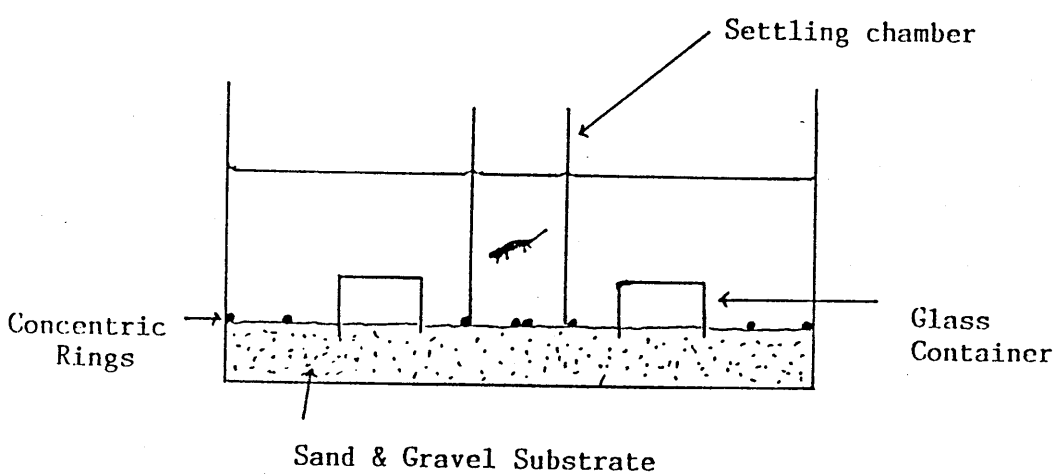


Figure 5.1 Apparatus used to test selective responsiveness based on visual cues alone.

Where two females were used to provide the subject with a choice of visual stimuli, they were selected to be of roughly equal size (±2.5mm snout-vent length) and wherever possible, of similar weight (±0.16g). As male *T. vulgaris* and *T. helveticus* of equal size could not be found, only those of each species with both well-developed external secondary sexual characteristics and a snout-vent length similar to their respective population means (40-41mm: *T. vulgaris*; 34-35mm: *T. helveticus*) were used. Eight series of trials were carried out (Table 5.1): four as a test of the hypothesis that newts show preferential selectivity to conspecifics of the opposite sex over heterospecifics when based on visual cues alone, and a further four to determine whether any preferences shown were due to avoidance of the heterospecific stimulus rather than an attraction to the conspecific stimulus. In the latter tests the arrangement was similar to those in the former, except that the conspecific stimulus was replaced by a blank control containing only water and an air pocket.

Series	Subject	Choice presented
1	Male *T. helveticus*	Female *T. vulgaris* v female *T. helveticus*
2	Male *T. vulgaris*	Female *T. vulgaris* v female *T. helveticus*
3	Female *T. helveticus*	Male *T. vulgaris* v male *T. helveticus*
4	Female *T. vulgaris*	Male *T. vulgaris* v male *T. helveticus*
5	Male *T. helveticus*	Blank v female *T. vulgaris*
6	Male *T. vulgaris*	Blank v female *T. helveticus*
7	Female *T. helveticus*	Blank v male *T. vulgaris*
8	Female *T. vulgaris*	Blank v male *T. helveticus*

Table 5.1 The trials carried out.

Twelve subjects chosen at random were individually tested for each series and prior to the start of the trial, each subject animal was allowed to settle in a large vertical opaque plastic tube occupying a central position within the tank. In line with the results from an earlier experiment

which suggest that a longer period is required by female *T. helveticus* than female *T. vulgaris* to recover from the effects of transfer, 20 minutes were allowed before removal of the tube for subjects of the former species and 15 for those of the latter. During the next 30 minutes the events in the aquarium were recorded on video. The first part of the trial was then repeated with the stimulus animals swapped over to control for position effects.

As the movement of the subjects was unrestrained, the ring system defined four regions on either side of the tank in which a subject animal could move. These were above the container, within 4.5cms (A), between 4.5 and 9cms (B), or greater than 9cms but within that half of the aquarium (C). The position of the snout was used as the criteria to denote the area occupied, but due to the limited amount of time spent by animals above the containers, only those data relating to the total time subjects occupied areas A - C are shown here. In order to determine whether positional bias or activity levels may have influenced the results, both the total time the subject spent in the left and right-hand side of the aquarium and activity indexes for each stimulus group were compared. The latter were calculated by drawing lines onto the video monitor screen to divide the containers into four equal imaginary sections. Tally counts of the number of times an animal crossed a line provided an index of general activity.

Males were judged to be sexually motivated if they courted one or both of the females presented to them. In those trials of series 1 and 2 where the males displayed, a comparison was made of aspects of male behaviour performed within 4.5cms of the containers. As waves could not be easily distinguished on the video recording, only whips and fans were scored as display acts. For all comparisons, the results of both parts of a trial

were combined. Due to some limited sample sizes, non-parametric descriptive statistics (median, 95% confidence limits, range) and statistical tests (Wilcoxon Matched-Pairs Tests) were used as appropriate. Although all trials were carried out as stated, many of the video tapes deteriorated before they could be analysed. All of those obtained for series 1 - 4 were intact, but only a limited number (between 5 and 7) of the trials for series 5 - 8 were of suitable quality for analysis.

Results

Of the four series of trials comparing the duration in which a subject remained in proximity to each female, significant preferences were observed in series 1 only (Table 5.2). In these trials, male *T. helveticus* remained for the longest with conspecific females in both of the regions within 9cms (i.e. A and B). Beyond 9cms distance, no significant differences were observed. The possibility that these results were largely influenced by either the level of activity of stimulus females, or by positional preference of subjects within the aquarium can be discounted, as no significant differences were observed between groups when both of these factors were tested. The data therefore indicate that male *T. helveticus* show an active preference for conspecific females over heterospecific females based on visual cues alone and, given the results of series 5 (Table 5.3) which indicates that there is no aversion to heterospecific females over the distance adopted during courtship (i.e. within 4.5cms., see pg.111), this further suggests that this preference is based on attraction to conspecific females, rather than avoidance of heterospecific females.

In seven of the 12 trials (58.3%) in which male *T. helveticus* were presented with a choice of females (series 1), the subjects were sexually motivated, as indicated by their display behaviour towards at least one of

(SERIES) REGION	DURATION IN PROXIMITY TO STIMULUS median (95% confidence limits)		WILCOXON* n T P
(1)	Consp. female	Heterosp. female	
A	479.9s (3.1-1275.1s)	59.3s (0.1-204.2s)	11 4 <0.01
B	145.2s (41.5-195.2s)	78.1s (36.0-139.7s)	12 10 <0.05
C	1335.0s (804.3-1713.5)	1275.8s (919.0-1738.1s)	12 31 >0.05
(2)	Consp. female	Heterosp. female	
A	370.4s (201.3-474.4s)	338.2s (67.3-654.0s)	12 38 >0.05
B	156.6s (70.5-360.7s)	173.9s (89.4-223.4s)	12 35 >0.05
C	1114.0s (758.7-1588.3s)	1225.0s (859.3-1714.1s)	12 37 >0.05
(3)	Consp. male	Heterosp. male	
A	68.8s (3.4-117.7s)	27.6s (0.0-72.2s)	11 16 >0.05
B	71.0s (27.6-215.1s)	60.9s (10.0-93.0s)	12 30 >0.05
C	1847.1s (892.2-2422.6s)	1522.5s (982.2-1792.4s)	12 28 >0.05
(4)	Consp. male	Heterosp. male	
A	154.0s (28.9-184.4s)	114.4s (70.1-183.6s)	12 28 >0.05
B	119.4s (23.4-313.6s)	136.8s (29.9-298.5s)	12 37 >0.05
C	1437.1s (1250.8-1957.0s)	1343.2s (1067.8-1809.1s)	12 29 >0.05

Table 5.2 Duration spent near conspecific and heterospecific visual stimuli. Series subjects: 1, male *T. helevticus*; 2, male *T. vulgaris*; 3, female *T. helveticus*; 4, female *T. vulgaris*. Distance from containers: Region A = ⩽4.5cms, B = 4.5-9.0 cms, C = ⩾ 9.0 cms. Abbreviations: Consp. = conspecific; Heterosp. = heterospecific; Wilcoxon = Wilcoxon Matched-Pairs Test corrected for ties; s = seconds.

the females (Figure 5.2). During these seven trials, the median duration of visits within 4.5 cms of the containers was 5.2 seconds longer for conspecific females than for heterospecific females (Table 5.4) and a significantly greater proportion of the total trial time, a higher frequency of visits and a higher number of whips and fans were performed towards conspecific females (Table 5.5). Although the paired females were selected for similarlity of size, statistical analysis showed that the female *T. helveticus* used in these trials were slightly smaller in length

(SERIES) REGION	DURATION IN PROXIMITY TO STIMULUS median (95% confidence limits)				WILCOXON* n	T	P
(5)	Heterosp. female		blank				
A	51.8s	(0.0-503.3s)	45.0s	(0.0-363.0s)	6	7	>0.05
B	15.1s	(0.0-239.4s)	111.8s	(0.0-699.4s)	7	1	<0.05
C	1036.7s	(346.3-2584.4s)	1638.9s	(923.1-2614.5)	7	6	>0.05
(6)	Heterosp. female		blank				
A	55.2s	(6.8-508.2s)	71.0s	(0.4-701.7s)	5		IS
B	33.5s	(5.3-185.7s)	27.9s	(10.6-639.9s)	5		IS
C	1629.5s	(999.5-1803.4s)	1685.6	(1264.7-1850.7s)	5		IS
(7)	Heterosp. male		blank				
A	25.3s	(0.0-113.1s)	27.3s	(0.0-207.1s)	5		IS
B	15.6s	(8.4-231.2s)	120.7s	(17.5-297.9s)	5		IS
C	1694.9s	(1051.0-2383.5s)	1652.4	(948.4-1990.3s)	5		IS
(8)	Heterosp. male		blank				
A	70.4s	(0.0-383.9s)	23.4s	(0.0-232.3s)	6	6	>0.05
B	14.4s	(8.4-231.2s)	49.7s	(0.0-101.7s)	6	9	>0.05
C	1830.7s	(1445.9-2641.1s)	1691.5	(228.0-1785.0s)	6	4	>0.05

Table 5.3 Duration spent near heterospcific and blank visual stimuli. Series subjects: 5, male *T. helveticus*; 6, male *T. vulgaris*; 7, female *T. helveticus*; 8, female *T. vulgaris*. Distances and abbreviations are the same as those listed in Table 5.1. except for IS = insufficient sample size for statistical analysis.

than female *T. vulgaris* (Wilcoxon Matched pairs Test: n=7, T=2, P<0.05), although there were no significant differences in weight. The preferences found in male *T. helveticus* cannot therefore, be attributed to a similar process of selective responsiveness for larger females as that observed by Verrell (1986b) in *T. vulgaris*. Although some courtship display was directed towards heterospecific females by male *T. helveticus*, the extent of heterospecific courtship was limited (Figure 5.2), which further suggests that males of this species visually discriminate between conspecific and heterospecific females when sexually motivated.

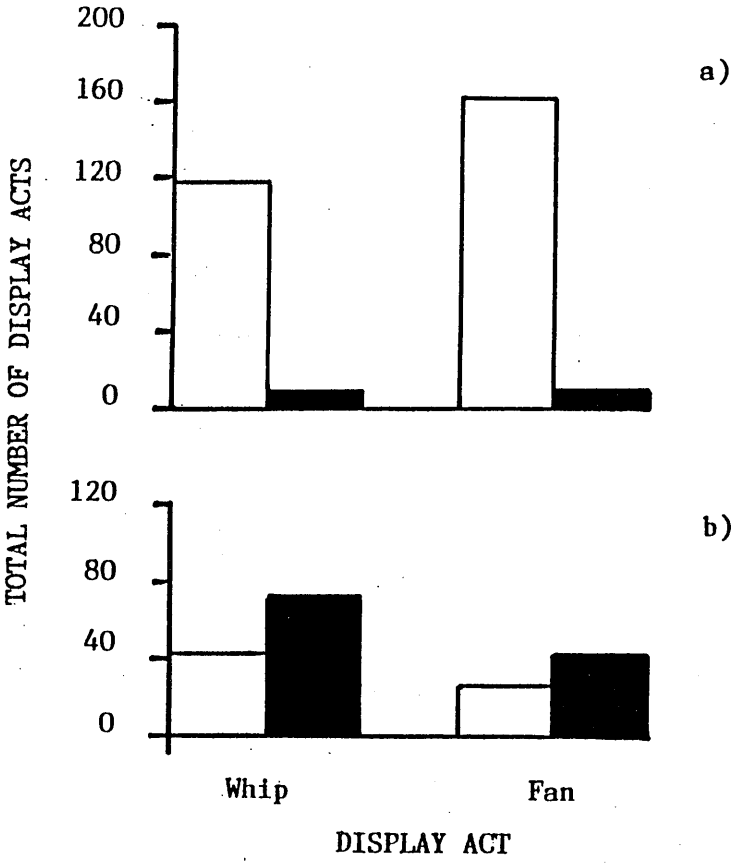


Figure 5.2 Total number of whips and fans performed. (Series 1 and 2) Where a) = male *T. helveticus* and b) = Male *T. vulgaris*; Open histogram = conspecific female, blocked histogram = heterospecific female.

SUBJECT	FEMALE	DURATION OF VISIT median	range
Male *T. helveticus*	conspecific	23.8s	0.1-655.6s
	heterospecific	16.6s	1.1-503.1s
Male *T. vulgaris*	conspecific	19.2s	1.6-429.1s
	heterospecific	18.7s	0.1-689.8s

Table 5.4 Duration of visits to conspecific and heterospecifc females by sexually motivated males. (Within a distance of 4.5cms).

	FEMALE	MEDIAN	RANGE	WILCOXON
Male T. helveticus:				
% Total time	Consp.	27.1	5.1 - 55.8	n=7, T=0
	Heterosp.	4.7	2.1 - 18.0	P<0.05
Number of visits	Consp.	13	5 - 38	n=7, T=0
	Heterosp.	7	1 - 16	P<0.05
Number of Wh & F	Consp.	27	3 - 131	n=7, T=0
	Heterosp.	1	0 - 9	P<0.05
Male T. vulgaris:				
% Total time	Consp.	11.1	4.0 - 21.4	n=8, T=12
	Heterosp.	11.1	0.8 - 30.6	P>0.05
Number of visits	Consp.	10.5	3 - 25	n=8, T=14
	Heterosp.	7.5	2 - 29	P>0.05
Number of Wh & F	Consp.	6.5	0 - 24	n=8, T=11
	Heterosp.	15.5	0 - 33	P>0.05

Table 5.5 A comparison of the behaviour of sexually motivated males within 4.5cms of conspecific and heterospecific females. Abbreviations: Consp. = conspecific; Heterosp. = heterospecific; Wilcoxon = Wilcoxon Matched-Pairs Test corrected for ties; Wh = whips; F = fans.

In contrast, male *T. vulgaris* showed no preferential selectivity for duration of proximity to females of either species (Table 5.1) and of those eight males which displayed, courtship was equally vigorous towards both conspecific and heterospecific females (Table 5.5). These data strongly indicate that male *T. vulgaris* do not show selective responsiveness towards either conspecific or heterospecific females when based on visual cues alone and further suggest that they lack the ability to visually discriminate between females of these two species, at least after a period in captivity.

Females of both species showed no significant preferential selectivity for remaining in proximity to either conspecific or heterospecific males during any of the trials which could be statistically analysed (Table 5.2

& 5.3). As only a limited number of transient courtship acts were attempted by a few restrained males, these results suggest that both female *T. helveticus* and female *T. vulgaris* do not actively discriminate between non-courting conspecific and heterospecific males when based on visual cues alone.

Discussion

The ability of *T. helveticus* to visually distinguish potential mates would presumably be most efficient where the light intensity is sufficient to provide the full range of visual cues. It would therefore be predicted that courtship in this species in generally not a noctural activity. The experiments in this section were carried out at a reduced light intensity which was sufficently high to permit colour distinction in humans and therefore, probably also in newts. This provided an analogous environment to that in the hours succeeding dawn and preceding dusk when courtship activity is most common in *T. vulgaris* (Dolmen 1983, Griffiths 1985) and it indicates that these experiments were a valid test of selectivity based on visual cues in this species. As an asymmetry exists, I must conclude on the basis of the results obtained here that visual cues form part of a specific mate recognition system for male *T. helveticus*, but not for male *T. vulgaris* and that preferential selectivity for conspecific females in the former group probably contributes to sexual isolation between the two species.

The results for female *T. helveticus* and *T. vulgaris*, taken at face value, suggest that they lack the ability to discriminate between conspecific and heterospecific males based on visual cues alone. However, as it is males and not females, which adopt the active role in pursuing a mate during courtship and as this experiment requires a subject to actively move

toward and remain with a stimulus type, it cannot be discounted that females recognise conspecific males on the basis of external morphology, but that they do not actively do so unless the stimulus is brought to them (i.e. such as when the male takes up his display position during a courtship encounter); neither can it be discounted that females show preferential selectivity for the differing movements carried out by the males of the two species during their respective display repertoires. It must therefore be concluded that an experiment where the stimulus is brought to the female may be more suitable for testing visual discrimination in females of these two species.

Despite the previous caveat, the data here lend no support to Halliday's (1977a) hypothesis that female selectivity has influenced the evolution of anatomical differences between male *T. vulgaris* and *T. helveticus* through character displacement. More recently, Halliday (in press) suggested that the diversification of courtship display movements between the different *Triturus* species occurred as a result of sexual selection through (conspecific) female choice and it is possible that the diversification of some epigamic features was a consequence of this change, not necessarily by taking the form of a direct visual signal that was more attractive to females, but as an adaptive feature for males which increased the efficiency of their display and enabled them to court females more vigorously or for longer. Males with such morphological adaptations would gain a selective advantage and the characters would spread and eventually become fixed in the population. This process may continue until the characters reach the limit of their development through physiological constraints internal to the male. Most significantly, it is a process which would be adaptive both in allopatry and sympatry and is therefore concordant with Pecio and Rafinski's (1985) observations relating to the

morphology and behaviour of allopatric *T. v. vulgaris* which they report show typically developed secondary sexual characteristics and courtship display.

OLFACTORY CUES.

That different systems of chemoreception contribute to reproductive isolation has been reported by several authors. Perrigon and Bronson (1982) report that incompatibilities in priming communication on an olfactory basis contribute to the reproductive isolation of deermice; olfactory recognition of species of mammals has been reviewed by Muller-Schwarze (1974). It is suggested that Sceloporine lizards taste foreign objects to identify species through chemoreception (Hunsaker 1962), whilst in tortoises, identification of conspecific females is based on olfactory investigation of the cloacal area (Auffenberg 1965). Specificity for conspecific female odour trails has been found in males from sympatric species of garter snakes (Ford & Schofield 1984), but not in allopatric species (Ford 1982). The importance of sex pheromones in the reproductive isolation of lepidopterous species has been reviewed by Roelofs and Carde' (1974): examples where pheromone specificity facilitates conspecific pair formation in moths includes studies by Collins and Tuskes (1979), Greenfield (1983), Honma and Tamaki (1976) and Sanders (1971). Lanier and Burkholder (1974) suggest that pheromone systems are probably only one of a number of isolating mecahnisms that function in various combinations between species of coleoptera. Such a limited contribution has been found in ticks, where female sex pheromones activate only conspecific males to detach from their host and search for females, but differences in courtship behaviour are reported to be the primary factor responsible for reproductive isolation (Andrews 1983).

Reviews of morphological, physiological and behavioural studies associated with amphibian olfaction have been published by Salthe and Mecham (1974), Madison (1977) and Halpern (1980) and it is thought that the conspicuous linkage between courtship movements, body contacts and the distribution and activity of specialised glandular tissue in urodeles is related to the exchange of chemical information (Madison 1977). Chemoreception is thought to be the dominant sense used by many urodele species to recognise the sex and species of potential mates (Arnold 1977, Madison 1977) and response to chemical signals between conspecific partners under differing experimental conditions has been reported in *Triturus* by numerous authors (Belvedere *et al.* 1988, Cedrini & Fasolo 1971, Malacarne & Vellano 1982, 1987; Malacarne *et al.* 1984, Margolis 1982, Prechtl 1951, Tinbergen & Ter Pelkwijk 1938, Verrell 1986b, Zippelieus 1948).

The importance of olfactory cues associated with species recognition in *Triturus* has been poorly studied. However, Arnold (1972) believes that chemical signals are of primary importance in species recognition in salamanders and a preference for the odour of conspecific partners over heterospecific partners has been observed in salamanders of the genera *Desmognathus, Taricha* and *Plethodon*, by Verrell (1989), Verrell and Arnold (1989), Davis and Twitty (1964) and Dawley (1984, 1986, 1987), respectively. Indeed, although both Halliday's (1977a) and my laboratory studies (Chapter 4) indicate that male *T. vulgaris* will court female *T. helveticus*, Halliday could not discount that some degree of interspecific discrimination based on olfaction may exist under natural conditions.

In this section I investigate the response of male and female *T. vulgaris* and *T. helveticus* to a choice of water-borne conspecifc and heterospecific odours presented by means of a Y-shaped olfactometer. A similar

arrangement for the testing of sex and species discrimination based on olfaction alone has been used successfully by others working on urodeles (Dawley 1984, 1986, 1987; Verrell 1985, 1986, 1989).

Method

Aquatic phase newts were obtained from various sites (Table 2.5) and were maintained under the same conditions as that described earlier (Chapter 1). The experimental apparatus consisted of two glass reservoirs linked to side-arm flasks containing the odour source, which were connected by lengths of tubing via a flow-inducer (peristaltic pump) to the arms of a Y-shaped perspex olfactometer (Figure 5.3). A black cardboard screen was placed between the flasks and the olfactometer to exclude potential visual stimuli from the odour source animals. Sets of three newts of the appropriate sex and species were used to provide the odour sources; aged tap water alone was used as a blank. Trials involving male subjects were carried out using sets of odour source females of roughly equal size (±1.5mm SV), but due to the difficulties faced in obtaining male *T. vulgaris* and *T. helveticus* of equal size, male newts of approximately average length for each species were used in trials involving female subjects.

All trials were carried out from March to June under dim lighting conditions in the early morning and late evening to correspond with the peaks of courtship activity indicated by field observations. At least half an hour before the trials began, all parts of the apparatus were filled with aged tap-water to pre-set levels and the odour source animals were placed in the side-arm flasks. Whilst water from each flask was pumped simultaneously into the appropriate arms of the olfactometer at rate of 100mls/90seconds, the subject was placed in the start-chamber to settle.

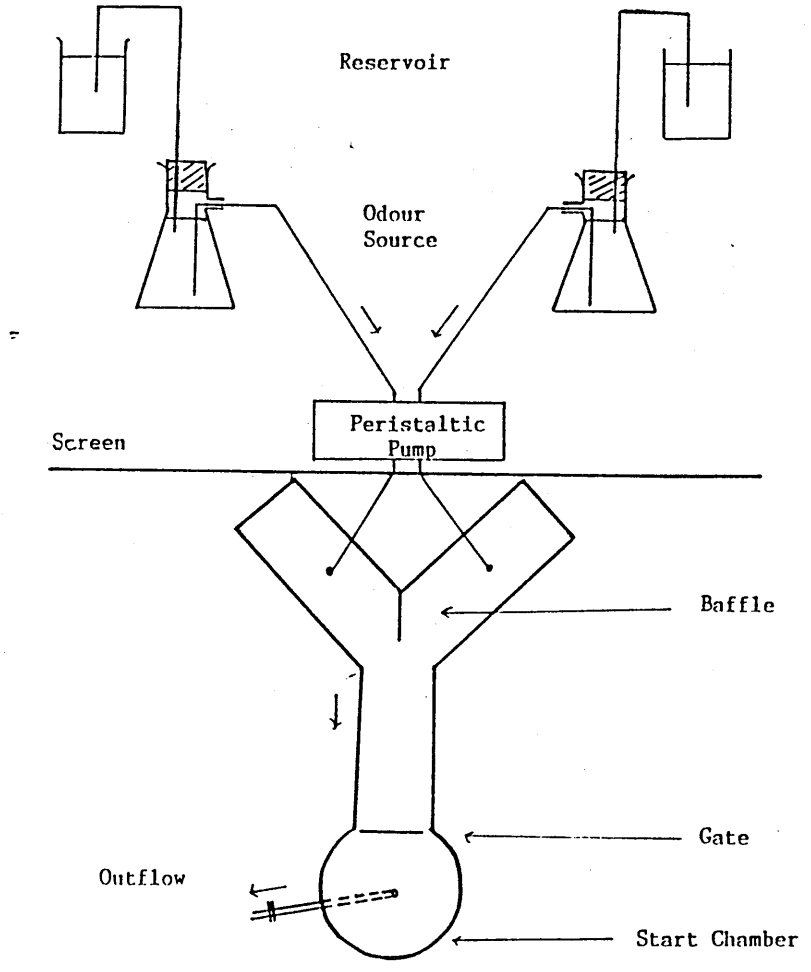


Figure 5.3 The olfactometer.

When the olfactometer was almost completely full of water, odour trails were established by releasing a continuous trickle of water from the start chamber outlet. Even mixing of water was aided by the use of a baffle and this, and the path of the odour trails, was confirmed by the use of coloured dyes. After a minimum settling period had elapsed of 10 minutes for *T. vulgaris* and 15 minutes for *T. helveticus*, the gate was removed and the subject was allowed 15 minutes to move freely into the maze. If this had not taken place in the allotted time, the trial was abandonded and repeated another time.

A note was made of the subject's pattern of approach to the 'choice' area by the baffle and also of sniffing, bucco-pharyngeal pumping and courtship display acts. A preference was registered when the subject moved into one of the arms of the olfactometer by at least a head-length; both the preferred odour and side (i.e. left or right) were recorded. The trial consisted of two parts, the second part was a repeat of the first with the same animal, after the apparatus had been drained and refilled with the position of the odour sources reversed. To control for position effects, the order of presentation of the odour combinations was alternated between trials in each of the three series carried out:-

Series 1: Newts of the four groups (male and female *T. vulgaris* and *T. helveticus*) were presented with conspecific and heterospecific odours of animals of the opposite sex to determine whether a preference for the odour of one species over the other would be exhibited in the absence of other sensory stimuli.

Series 2: Male newts of both species were presented with heterospecific female odour and a blank to determine whether any of the preferences exhibited were due to an avoidance of heterospecific female odour, or an attraction to conspecific female odour.

Series 3: Male and female *T. vulgaris* and male *T. helveticus* were presented with conspecific male and female odours to determine whether a preference existed for the odour of conspecifics of either sex.

The number of animals used in each series is shown in Table 2.1. All trials planned for series two and three could not be carried out due to difficulties in obtaining newts, particularly female *T. helveticus*.

The results of the odour preferences (i.e. odour A twice, odour A once and odour B once, odour B twice) and position preferences (i.e. left twice, left once and right once, right twice) for subjects in each group were tested for significance using a 3x1 Chi-squared test using Yates' Correction, based on a probability of 25%, 50% and 25%, respectively.

Results

The response of the subjects whilst in the olfactometer varied greatly. Some swam through the maze without changing sides, whilst others walked in a zig-zag fashion across the odour trails. Sniffing and bucco-pharyngeal pumping were observed in many subjects prior to reaching and directly in front of the baffle. In a small number of trials in each series, one or both of these behaviours were performed on each side of the baffle before an odour preference was registered.

Series 1: Male *T. vulgaris* and *T. helveticus* exhibited a significant preference for the odour of conspecific females over heterospecific females (which was strongest in the latter group), but females of both species no significant preference (Table 5.6A). A small number of male (n=5) and female (n=4) *T. vulgaris* stopped at the baffle and performed sniffing or bucco-pharyngeal pumping behaviour before choosing the conspecific odour. One male *T. vulgaris* performed fan display during both parts of the trial.

Series 2: Neither male *T. vulgaris*, nor male *T. helveticus* showed a significant preference for heterospecific odours over water alone (Table 5.6B). One male from each group tested the odours at the baffle; they both preferred the blank. Courtship behaviour was not observed during this series of trials.

A) Interspecific odour preference:

GROUP	SEX	N. TRIALS	PREFERENCE C	T	H	CHI-SQUARED TEST X^2	P
T. vulgaris	M	24	12	9	3	6.60	<0.05
T. helveticus	M	20	12	8	0	12.72	<0.01
T. vulgaris	F	20	5	12	3	0.72	>0.05
T. helveticus	F	24	6	16	2	1.77	>0.05

B) Heterospecific odour preference:

GROUP	SEX	N. TRIALS	PREFERENCE* H	T	W	CHI-SQUARED TEST χ^2	P
T. vulgaris	M	7	2	3	2	0.07	>0.05
T. helveticus	M	20	4	12	4	0.32	>0.05

C) Intraspecific odour preference:

GROUP	SEX	N. TRIALS	PREFERENCE* M	T	F	CHI-SQUARED TEST χ^2	P
T. vulgaris	M	20	5	8	7	0.72	>0.05
T. helveticus	M	20	4	11	5	0.12	>0.05
T. vulgaris	F	24	7	15	2	2.60	>0.05

Table 5.6 (A-C) Discrimination based on olfactory cues alone. * Where: C=conspecific odour chosen twice; H=heterospecific odour chosen twice; T=both odour types chosen once; W=water blank chosen twice; M=conspecific male odour chosen twice; F=conspecific female odour chosen twice.

Series 3: No significant preferences were observed for the odours of conspecific animals of the same sex over conspecific animals of the opposite sex in any of the groups (Table 5.6C). However, six of the seven male *T. vulgaris* and four of the six female *T. vulgaris* which performed sniffing or bucco-pharyngeal pumping behaviour at the baffle preferred the heterosexual conspecific odour. Courtship behaviour was performed during one trial by a male *T. vulgaris*.

No significant side bias (P>0.05) was observed in any of the series.

Discussion

The results indicate that male newts show some olfactory discrimation between conspecific and heterospecific females and suggest that the preference shown by male newts for the former is due to an attraction for conspecific female odour, rather than an avoidance of heterospecific female odour. Research by Verrell (1985, 1986b) on *Notophthalmus viridescens* and *T. vulgaris* suggests that male newts can assess the size of female newts using olfactory cues and that they prefer larger females. Nevertheless, the preferences of male *T. vulgaris* and *T. helveticus* observed here cannot be attributed to differences in size, as the two sets of females to whose odour they were exposed were equally matched.

In contrast to Verrell's (1986b) findings that male *T. vulgaris* prefer the odour of conspecific females, but not males, over plain water, the results in Series 3 suggest that males of both species are neither attracted nor repelled by the odour of conspecifics of either sex and that they possibly produce an odour which is general to the species. The trials in Series 3 were carried out in late May and June after what is thought to be the most intense periods of courtship and egg-laying (Verrell & Halliday 1985, Verrell *et al.* 1986, Verrell & McCabe 1988) when the reproductive activity of both male and female newts may have been in decline. This discrepancy with Verrell's (1986b) data may therefore have arisen because some of the newts used in Series 3 were sexually less receptive than normal.

Evidence that reproductive activity in some male *T. vulgaris* had not totally declined was observed when two males of this species displayed whilst in the olfactometer. Although the odour of conspecific females was present during both of these trials and no courtship behaviour was observed in the absence of conspecific odour, I can merely conclude that

courtship behaviour in male *T. vulgaris* may be elicited in the presence of olfactory cues alone.

Female *T. vulgaris* and *T. helveticus* showed no significant preferences in any of the three series. However, this should not be accepted blindly as an indicator that female newts are unable to discriminate between the odour of conspecific and heterospecific males either prior to, or during, courtship for several reasons. First, during courtship encounters it is the male which initially orientates toward the female and her first 'positive' response is to remain static; approaching is usually the secondary response of a sexually motivated female. It might be concluded that in an experiment of this design only a more highly motivated female may test the environment and approach a preferred odour source and that less motivated females may move randomly. Secondly, chemical information may only be released or transferred by male newts during courtship itself. Cedrini and Fasolo (1971) found that female *T. (cristatus) carnifex* show chemosensory activity towards water inhabited by sexually activated males. They suggested that the dorsal (abdominal) gland, which increases in size during the aquatic phase and is therefore probably active during the reproductive season (Verrell *et al.* 1986), might be the source of olfactory stimuli emitted by male newts during courtship. Further evidence that pheromones are released from the dorsal gland of male Crested newts is provided by Malacarne *et al.* (1984) and Malacarne and Vellano (1987); one of these pheromones is known to include progesterone in *T. carnifex* (Belvedere *et al.* 1988). Transfer of the products of the dorsal gland probably takes place when water is wafted towards the female's snout during fan bouts. Some evidence to support the hypothesis that females are attracted to conspecific odours produced during courtship has been obtained by Belvedere *et al.* (1988), who found that female *T. italicus*, *T.*

alpestris and *T. carnifex* were attracted to courting conspecific pairs by olfactory cues alone.

The design of this experiment may be a more suitable test of odour discrimination in male newts than in females. Future experiments of this nature may be more reliable if trials are carried out earlier in the breeding season before egg-laying commences, when females are more likely to be sexually responsive.

Further Analysis Of News Showing Evidence Of Olfaction

Margolis (1982) concluded that bucco-pharyngeal movements were analogous to sniffing behaviour and hence, both behaviours can be accepted as evidence of olfaction. As observations here suggest that newts which performed one or both of these behaviours during a trial showed some discriminating ability, the response of these newts and those that showed no evidence of olfaction, is further investigated.

Method

Using the results of the earlier trials, a comparison was made between the response of groups of animals in each series which sniffed and/or performed bucco-pharyngeal pumping behaviour with those that did not perform either of these behaviours. Where sample sizes were adequate (>6), a Sign Test was carried out for each group to determine whether significant preferences were observed.

Results

The results are shown in Table 5.7. Sample sizes of animals performing sniffing or bucco-pharyngeal pumping behaviours were generally low and emerged for this category: 81% of these animals preferred the odour of

conspecifics over heterospecifics in Series 1 and 69% preferred the odour of conspecifics of the opposite sex over conspecifics of the same sex in Series 3. A significant preference for the odour of conspecific newts of the opposite sex was found in Series 1 for male and female *T.vulgaris* which showed evidence of olfaction and also male *T. helveticus* which did not show evidence of olfaction. With the exception of the female *T. helveticus* group in series one which did not perform behaviour associated with olfaction that was excluded from testing due to low sample size, random preference was observed in all other categories and groups, including those in Series 2 and 3.

SERIES	GROUP	FREQUENCY OF RESPONSE: Olfaction			No Olfaction		
One		CO	HO	P	CO	HO	P
	Male T. vulgaris	20	5	<0.05	13	10	>0.05
	Male T. helveticus	7	2	>0.05	25	6	<0.01
	Female T. vulgaris	8	0	<0.01	14	18	>0.05
	Female T. helveticus	4	1	SI	24	19	>0.05
	Total	39	8		76	53	
Two		W	HO	P	W	HO	P
	Male T. vulgaris	2	4	>0.05	5	3	>0.05
	Male T. helveticus	4	2	>0.05	16	18	>0.05
	Total	6	6		21	21	
Three		CS	CO	P	CS	CO	P
	Male T. vulgaris	5	9	>0.05	13	13	>0.05
	Male T. helveticus	2	7	>0.05	17	14	>0.05
	Female T. vulgaris	8	17	>0.05	11	12	>0.05
	Total	15	33		41	39	

Table 5.7 Discrimination in newts showing evidence of olfaction.
Response abbreviations: CS = conspecific, same sex; CO = conspecific, opposite sex; HO = heterospecific, opposite sex; W = water blank; SI = sample size inadequate for Sign Test.

Discussion

These results support the conclusions drawn earlier, but with one notable exception: female *T. vulgaris* which actively test the environment show some discrimination in favour of conspecific males when provided with olfactory cues alone. These results clearly recommend that the trials for females in Series 1 are particularly worthy of repetition earlier in the breeding season, with emphasis placed on the response of subjects showing evidence of olfaction. Somewhat more surprising however, are the data for male *T. helveticus* in Series 1 which showed no evidence of olfactory behaviour. Males of this species are probably able to discriminate between odours by more subtle means than sniffing or bucco-pharyngeal pumping and it is also possible that they may have a more highly developed olfactory discrimination ability than other forms.

The water-borne odours produced by the newts here are clearly long-lasting chemical signals that act at a distance and are effective when diluted. Olfactory cues are more persistent than visual cues and may be transferred through patches of aquatic vegetation which may obscure visual signals (Smith 1973). They may assume greatest importance at night when vision is poor or in eutrophic ponds with turbid water. Olfactory cues may also be important to female newts in the earliest weeks of the breeding season when the external secondary sexual characteristics of males are less well developed (see Chapter 2).

Liley (1982) reported that in many fishes, odours may serve two functions, both to attract a partner and/or to stimulate courtship. Arnold and Houck (1982) addressed this issue and made a distinction between chemical signals which are effective prior to courtship, acting at a distance to attract one sex to another (sex pheromones) and those that are delivered

directly to a partner after the initial encounter but before sperm transfer, which increase the mating success of one or both partners (courtship pheromones). The chemical cues tested in this experiment clearly fall into the category of sexual attractants, but without further experimentation, it cannot be discounted that the same odour produced prior to courtship may also act as a stimulant during courtship, or that more than one chemical may be produced which may serve in either capacity.

The sexual attractants produced by *T. vulgaris* and *T. helveticus* appear to act directly to increase the probability of conspecific partners meeting which suggests that they are a part of a system of mate recognition. Whether olfactory cues increase the probability of conspecific partners mating successfully once courtship has been initiated, or whether they decrease the incidence of heterospecific courtship and mating success between these two species has not yet been fully evaluated. Dawley (1986), working on terrestrial salamanders, found that female *Plethodontid glutinosus* were repulsed by male *P. aureolus* odours, hence it is possible that chemical signals produce an avoidance of heterospecific partners under natural conditions, possibly acting synergistically with other sensory cues, but there is currently no evidence to indicate that this occurs in *Triturus*. Alternatively, as the work of Gwynne and Morris (1986) on katydids and Verrell (1989) on salamanders suggests, sexual isolation may also be brought about if one sex fails to respond to the chemical cues produced by a heterospecific partner. The evidence in this section suggests that the latter alternative probably contributes to sexual isolation between *T. vulgaris* and *T. helveticus*, wherein both sexes respond only to conspecific odours.

TACTILE AND VISUAL CUES - THE FAN DISPLAY.

In previous chapters I have presented evidence of interspecific differences between males and have suggested in agreement with other authors (e.g. Halliday 1975b, 1977a; Wambreuse & Bels 1984), that during courtship and fan display particularly, the male may be transferring information by several different modalities which may play a role in sexual isolation. These include visual cues of movement and tactile cues from the water current produced. It is by means of the lateral line organs situated predominantly around the head and also on the flanks (Smith 1973), that newts are able to detect small movements and pressure changes in the water.

In this chapter I investigate female preference for the fan display of male *T. v. vulgaris* and *T. h. helveticus* using an artificial tail model set at the speeds described by Halliday (1975b) and to the amplitudes described in Chapter 3. The model provides potential cues simultaneously via two modalities (tactile vibrational cues and visual cues of movement): these cannot be separated. Duration of proximity to the male, number of positive orientation acts performed toward the male and distance over which the females approach the male are all acceptable indicators of female receptivity and these have been used to investigate selectivity.

Method

Laboratory experiments were carried out at room temperature (≈20°C) between the hours of 15.00 and 22.00 GMT in May and early June 1984 using aquatic phase animals captured and maintained under the condition stated in Chapter 1. Illumination was provided by dim spotlights and low-intensity angle-poise lamps. The apparatus consisted of an aquarium with a fine sand and gravel substrate overlaid with a $5cm^2$ grid, that was

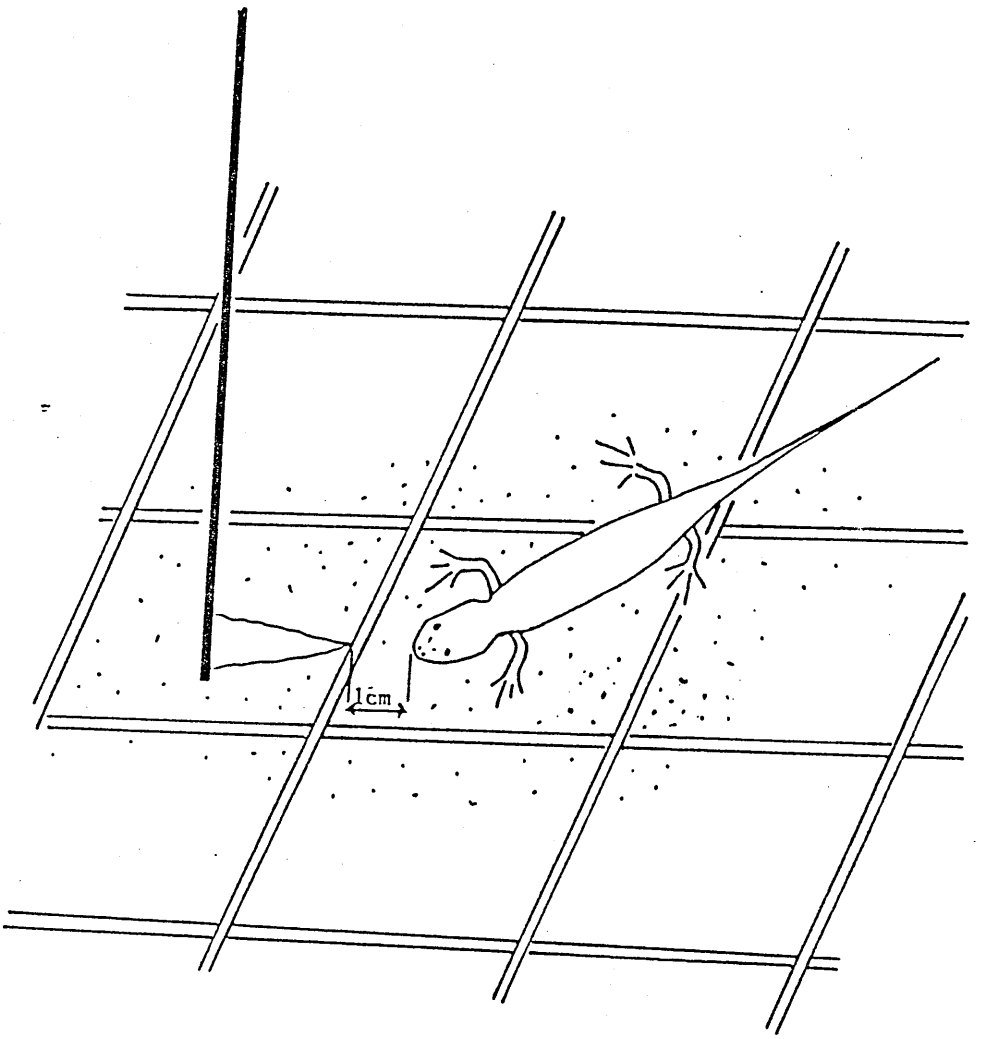


Figure 5.4 The artificial male tail mimic.

filled to a depth of 15cms with aged tap water; and a fine metal rod with a triangular, 2.5cm long and 1.25 cm deep, colourless, semi-transparent, flexible, laminar plastic tail mimic attached to the lower end with a variable-beat, hand-held motor at the other (Figure 5.4). By altering the controls of the motor, the frequency and amplitude of the tail model were adjusted to 6 Hz and an amplitude at the tip of 1.5cms, or 12 Hz and an amplitude of 1cm, to correspond with the respective fan display pattern of male *T. vulgaris* and male *T. helveticus*.

Preliminary tests with ink indicated that the model did not sustain a directional stream of water over the distance maintained by a male newt.

To ensure that the water current produced by the model reached the female's lateral-line organs, it was positioned during trials immediately above the substrate at a distance of 1cm from the snout, at an angle of 45° from the mid-line axis of the head. As a consequence, the distance from the model tail tip to the female's snout was nearer than that usually observed during normal courtship encounters (ref. pg. 111).

After 20 minutes settling time in the aquarium, twenty females of each species were individually tested with the model six times during a trial, alternating between conspecific and heterospecific display mode presentations to control for order effects. A one-minute interval was allowed between the end of one presentation and the beginning of the next. Each presentation was as follows:-

Once the female was stationary, the tail model (beating in the appropriate display mode) was lowered into the water on the opposite side of the tank before being brought slowly into position. The duration over which the female remained in the vicinity of the tail was measured to the nearest second using a stopclock and in addition, the distance over which a female positively advanced (+Adv) towards the tail was estimated using the substrate grid as a guide and the tail was moved backwards the same distance. The female's actions were simultaneously recorded onto cassette tape and were later transcribed to the nearest second onto paper. A presentation was defined as having ended when the female moved her head away from the tail by more than 5cms, or when a female ascended to the surface to breathe and did not immediately return, or after five minutes had elapsed, whichever was the sooner. If the female ascended for air, the stopclock was stopped on ascent and only re-started if she returned directly to face the tail within a distance of 5cms of her snout. The

response of the female in the three presentations for each display mode were combined and a comparison was made of response to the conspecific and heterospecific male display modes for proximity duration, number of positive responses (e.g. Or, +Adv), total distance moved toward the tail (+Adv) and rate of bucco-pharyngeal pumping bouts, using Wilcoxon Matched-Pairs Tests corrected for ties. Correspondingly, non-parametric descriptive statistics (median ± 95% Confidence Limits) are provided.

After the six presentations had been carried out a five minute interval was allowed before a conspecific male was placed in the tank with the female for 30 minutes. Sequential interactions between the partners during this period were noted to gain some indication of the sexual receptivity of the females. Where courtship occurred, the number of spermatophores deposited by males in response to positive behaviour by females which had previously approached the tail mimic were compared to those that earlier had not. Sample sizes of courting pairs were too low to allow meaningful statistical analysis of these data.

Results

Female behaviour in the presence of the tail mimic was generally in accordance with normal response to a courting male and ranged for both species between negative responses such as movement away from the tail to positive responses including sustained positive advance. However, two female *T. vulgaris* lunged and snapped once each at the tail: one in response to the conspecific mimic and the other to the heterospecific mimic. Responses such at these are more typically associated with feeding behaviour than courtship behaviour and they indicate that a small proportion of female *T. vulgaris* related to the model as a prey item rather than a courtship stimulus.

BEHAVIOUR	FEMALE		FEMALE RESPONSE TO TAIL MIMIC FAN MODES § Consp.	Heterosp.	WILCOXON M-P TEST n	T	P
Proximity Duration (secs)†	*T. vulgaris*	Median	50.0	62.0	20	36	<0.01
		95%CL	31-79	30-94			
	T. helveticus	Median	190.5	95.0	19	36	<0.05
		95%CL	82-269	46-163			
Positive Acts †	*T. vulgaris*	Median	2	2	13	37	>0.05
		95%CL	0-4	0-4			
	T. helveticus	Median	1	0	20	36	<0.05
		95%CL	0-1	0-2			
Distance Approached (cms)#	*T. vulgaris*	Median	5.5	12.0	10	18	>0.05
		95%CL	1-32	0-46			
	T. helveticus	Median	13.5	1.5	4		(SI*)
		95%CL	1-100	0-6			
Rate of Buccal Pumping Bouts †	*T. vulgaris*	Median	5.7	5.0	18	75	>0.05
		95%CL	2.9-8.6	2.0-11.2			
	T. helveticus	Median	9.0	7.1	20	102	>0.05
		95%CL	6.7-10.6	5.8-12.0			

Table 5.8 The response of female *T. v. vulgaris* and *T. h. helveticus* to a tail mimic set to conspecific and heterospecific male fan display modes. Key to symbols: § = Fan mode (*T. vulgaris* = 12 Hz and amplitude of 1.5cms; *T. helveticus* = 6 Hz and amplitude of 1cm). † = includes data from all trials. # = excludes data from females which did not approach tail mimic. * = SI: sample size inadequate for statistical test.

The results of the statistical tests are presented in Table 5.8. Proximity duration was longest in the presence of the tail mimic set to the faster fan mode for females of both species, although the response of female *T. helveticus* was the more highly significant of the two. The number of positive responses of female *T. helveticus* toward the conspecific fan mimic was also higher than that toward heterospecific fan display, but no significant differences were observed in the number of positive responses performed by female *T. vulgaris*. Of the 20 subjects tested for each species, 50% of the female *T. vulgaris* and 20% of the female *T. helveticus*

approached (+Adv) the tail mimic during at least one of the presentations. The female *T. helveticus* sample size for distance approached was too low to test significantly with confidence, but all four females approached the conspecific tail display over a greater distance than the heterospecific tail display (greatest difference = 97cms). However, no significant differences were found in the approach distances of female *T. vulgaris* toward fan display of either species. Rates of bucco-pharyngeal pumping bouts for both female *T. vulgaris* and *T. helveticus* were also not significant, which suggests that females of both species olfactorily test the environment to the same extent when presented with only the visual movement and tactile cue components of conspecific and heterospecific male fan display.

Males courted conspecific females which had previously responded to the tail mimic in 70% of subsequent pairings for *T. vulgaris* and 75% of subsequent *T. helveticus* pairings. The corresponding data for females that had failed to performed earlier positive advance behaviour were 10% and 87.5%, respectively. A comparison of the success to spermatophore deposition between the two categories based on earlier female response is not feasible for *T. vulgaris* due to the very low proportion of males which failed to court females in the latter category, but the data for *T. helveticus* are sufficient to suggest that there was a tendency for conspecific courtship to result in spermatophore deposition in a greater proportion of trials if females had earlier approached the tail than if they had not (Table 5.9). Therefore, females of the former category were probably of a slightly more highly sexually motivated state during the trials than those of the latter.

EARLIER RESPONSE		SPERMATOPHORES ELICITED DURING CONSPECIFIC COURTSHIP TRIALS *T. vulgaris*	*T. helveticus*
+Adv	n courtship trials	7	3
	% involving S↓	29%	100%
	n S↓	0-2	2-6
No +Adv	n courtship trials	1	14
	% involving S↓	0%	64%
	n S↓	0	0-5

Table ~~Figure~~ 5.9 **Number of spermatophores deposited in response to positive female behaviour by females which earlier approached the tail mimic compared to those that did not.**

Discussion

The behaviour of the females during the trials not only suggested that sensory information produced by the artificial tail broadly reproduced aspects of the sensory information provided during natural courtship, but also that the simplistic nature of the model probably produced a crude array of signals associated with other aspects of life. In the absence of the full complement of visual and possibly olfactory signals, some of these were probably interpreted as belonging to potential prey. The relationship between sensory cues produced by live food items and those produced during a courtship encounter probably share many similarities and may not be as erroneous as first appears (ref. wiggle - Chapter 3).

The fan display is clearly a primary mechanism for providing stimulation via several possible modalities by which a male persuades a female to remain in his vicinity and to mate; the faster tail beat of the male *T. helveticus* mimic may have provided greater stimulation than the slower *T. vulgaris* tail beat mimic, which more effectively decreased the initial tendency of females to move away. Using more modern technology than that available to Halliday (1975b), Raxworthy (1989a) found that the tail beat

of the two species at 12-14°C more closely approximated to 5.7 Hz (±0.22SD) for *T. vulgaris* and 9.33 Hz (±0.9SD) for *T. helveticus*. Speed of fanning (but not amplitude) is influenced by temperature, where higher temperatures result in faster fanning speeds (Raxworthy 1989a). The discrepancy between the two authors is probably because Halliday's experiments were carried out at a higher ambient temperature than Raxworthy's. In the field study described in Chapter 1 (which was carried out in May), the water temperature averaged around 14°C, hence it is probable that during most of the breeding season, fan speeds in the natural environment follow more closely those found by Raxworthy. However, the preference shown by female *T. vulgaris* cannot be attributed to the discrepancies in fan speed reported by these two authors. It is apparent that the components of fan display presented here do not form part of a specific mate recognition system for female *T. vulgaris*; information transferred during the whip display (the most common *T. vulgaris* display act), or sensory stimulation not provided by the mimic (such as odours) may make a more significant contribution.

THE IMPORTANCE OF VISUAL, TACTILE AND OLFACTORY CUES - AN OVERVIEW.

The widespread belief that sexual isolation in *T. vulgaris* and *T. helveticus* is brought about predominantly by female discrimination based on differences in male display can be largely rejected on the evidence of this and the previous chapter. It is apparent that selective responsiveness in males *T. vulgaris* and *T. helveticus* is important and there is growing evidence to suggest that this is probably common in other urodele species (e.g. Dawley 1986, Verrell 1989). Female selectiveness is most likely to be based on a combination of sensory modalities transferred during display which act to stimulate the female; individual heterospecific cues may be more, or less stimulatimg than

conspecific cues, but the data in this thesis suggests that overall, heterospecific cues are generally inadequate to persuade a female to mate.

I propose that under natural conditions, sexual isolation is probably a consequence of a dual system whereby primarily, the incidence of heterospecific courtship is limited by a male's failure to recognise (and be attracted to) the olfactory and/or visual cues produced by a heterospecific female. Secondarily, where interspecific courtship does occur, the display of the male may be enough to produce some positive behaviour in the female, but that the accumulated effects of display are not sufficient to raise the level of female receptivity further, or to maintain a sustained positive response. Over time there is a spiral of negative feedback in which a combination of inadequate response from the female, limited air supply and possibly lack of reinforcement due to the presence of inappropriate visual or olfactory cues from the female, leads to a reduction in the vigour of display of the male; this in turn reduces the level of stimulation transferred to the female and via a fall in the rate of accumulated stimulation, eventually leads to the breakdown of the encounter. The evidence suggests that it is not the form of male display in itself that is crucial to this process, but the olfactory and tactile cues transferred as a consequence of the acts performed. It is possible that the colour and patterning of the male are also important for stimulating the female, but there is currently only circumstantial evidence to support this.

At this point must be addressed the question of how these components of sexual isolation evolved? It would appear that assymetry exists between the species in the morpholgy, behaviour and preferences of the sexes and

this suggests that a number of different selection pressures have influenced the evolution of the forms.

It is possible that both male and female odours diverged in allopatry as a consequence of adaptation to local habitats. Males which developed the ability to locate females on the basis of odour would have an adaptive advantage over those that did not. They would succeed in finding and inseminating more females and thus produce more offspring, some of which would inherit this capacity. Ultimately this ability would spread and become fixed in the population. On return to sympatry, female odour may have diverged sufficiently for them to no longer be attractive to heterospecific males. A similar process could have brought about the preferential selectivity of male *T. helveticus* for conspecific females based on visual cues.

Sexual selection through female mate choice has probably contributed to the evolution of the highly developed secondary sexual characters and differing odours of males, whereby chance (genetic) differences in initial populations were magnified until constrained by physiological limitations and possibly also natural selection. Males which possessed such heritable qualities would succeed in producing both a greater number of and more attractive offspring, whilst females which mated with the most attractive males, would tend to produce attractive sons. Preferences shown by females of the two species may evolve independently in isolated populations, or in allopatry and on return to syntopy, or sympatry, heterospecific females may be exposed to cues which do not take the preferred form: these would consequently provide inadequate stimulation to persuade them to mate and so contribute to the sexual isolation of the two species.

The similarity in courtship display between *T. v. vulgaris* and *T. h. helveticus* is probably consequent on common ancestry and the dependance of both species on water as a medium for transferring both stimulation and spermatophores - a common solution to the same problem. The minor differences in the behaviour of the two species would probably have evolved in response to differing local habitats, physiology and competition. Sexual selection through female mate choice is likely to have played a part in shaping the male's courtship repertoire, although some of the evidence is this thesis suggests that many of the display acts have evolved primarily to facilitate spermatophore transfer and improve the efficency of mating. Like the other visual, olfactory and tactile differences investigated here, the differences in courtship and morphology that exist are unlikely to have arisen directly to reduce interspecific matings. It would appear that sexual isolation arises between *T. v. vulgaris* and *T. h. helveticus* largely as a by-product of the slightly different process that has evolved in each species to enable a male newt to locate, stimulate and transfer spermatophores as efficiently as possible to a conspecific female.

CHAPTER 6

CONCLUSIONS AND AREAS FOR FUTURE RESEARCH

Conclusions

Chapter 1 - Isolating Mechanisms.

* Male *T. h. helveticus* were observed attempting to court conspecific females in the field throughout the daytime; a small peak was observed at dusk, but no courtship was observed at dawn despite the presence of females. Actual sex ratios observed were heavily biased towards males.

* T. v. vulgaris and *T. h helveticus* commonly occur in the same ponds in Britain and they occupy similar feeding and microhabitat niches. Their courtship periods appear to overlap extensively. This suggests that seasonal and habitat isolation can be discounted as important contributing factors maintaining their reproductive isolation.

* Heterospecific courtship was not observed in the field between *T. v. vulgaris* and *T. h. helveticus* which suggests that males tend not to initiate interspecific courtship encounters.

* Mechanical isolation does not appear to operate - harnessed females picked-up conspecific and heterospecific spermatophores equally well.

* Laboratory-reared F1 *T. h. helveticus x T. v. vulgaris* hybrid larvae are capable of survival beyond metamorphosis. Hybrid mortality was greatest at the egg stage. Lower hatching success (when compared to purebred larvae) was found which suggests that gametic or zygotic mortality is extensive but not absolute.

* On the basis of combined evidence, I suggest that if heterospecific matings occur in the field, then some of the larvae produced would be potentially viable enough to survive to adulthood. Lack of natural hybrids (larval or adult) in the field indicates that interspecific matings are

rare. Sexual isolation mechanisms appear to be the most important contributing factor to reproductive isolation.

Chapter 2 - External Morphology

* Purebred *T. v. vulgaris* and *T. h. helveticus* efts may be distinguished on the basis of four distinct vertebral stripe characteristics. This technique is unsuitable for distinguishing hybrids, which show no unique morphological characters or consistent intermediate characters.

* Extensive sexual dimorphism occurs between male and female *T. v. vulgaris* and *T. h. helveticus*. Males are more highly coloured (differences were found in body and belly colours and size, density of pigmentation and number of spots), have crests and hind limb webbing (*T. h. helveticus*) or folds (*T. v. vulgaris*) and better developed tail fins and filament (*T. h. helveticus* only). Females of the two species are more cryptic in colouration and are roughly similar in size with male *T. v. vulgaris* being larger and male *T. h. helveticus*, smaller. Adult F1 hybrids between these two species have intermediate morphology.

* The four groups are separated by and may be distinguished mathematically with accuracy using a Discriminant Function analysis.

* The most and least suitable characters for distinguishing the four forms are described: fewer suitable categories exist when comparing females than for any other group.

* The upper mandible membranes that enclose the sides of the mouth in males and females, probably serve to improve the efficiency of feeding and have no direct reproductive function.

* Significant variation was found between two populations of *T. v. vulgaris* from Oxford and Littleworth Common in body dimensions and weight, but not in spot numbers or the size of male secondary sexual characteristics.

* Male *T. v. vulgaris* from populations syntopic and allotopic with *T. h. helveticus* showed equivalent relative secondary sexual development at the same stage in the breeding season.

* There is seasonal development of integumental characters in both males and females; peak development is reached in May. Secondary sexual characters in males include the crest, tail fin, hind limb webbing, filament (*T. h. helveticus* only) and the forelimb digits. The latter probably facilitate courtship by improving the male's grasp on the substrate, thus enabling him to maintain his display position more effectively.

* Female *T. h. helveticus* show seasonal development in tail filament length.

* It is suggested that most male integumental characters which seasonally develop (i.e. not only crests) increase the efficiency of mating by improving hydrodynamic flow between partners, or by acting as an auxilliary respiratory surface.

* There are many morphological differences between the forms on which species recognition (attraction) by males or females may be based.

Chapter 3 - Courtship behaviour.

* The courtship behaviour of *T. h. helveticus* is described; in overall plan it is broadly similar to that of *T. v. vulgaris*.

* The categories of orientation acts have been extended with the addition of Head Turn and Swivel. Based on male response, a distinction has been made between Positive Advance and behaviour where the female simply moves forward (Advance).

* *T. h. helveticus* wiggles are quantitatively and qualitatively described. They are an act which may be performed independently of others, although they are most closely associated with fanning and braking. A long latency

is allowed by the male before the performance of his subsequent act; in the interval between the two there is usually sufficient time for the female to respond.

* The wiggle influences the sequential but not the temporal outcome of the female's behaviour. It increases the probability of a transition from unresponsive or weakly unreceptive behaviour to positive behaviour. A positive motivational state in the female appears to be reinforced after the performance of a wiggle.

* A wiggling tail probably acts as a food mimic.

* Considerable variation is found in the performance of male actions between both phases and sequences of a courtship encounter.

* Push-back functions directly to improve the probability of spermatophore pick-up.

* Fan bouts are performed at a more obtuse angle, greater distance, faster speed and smaller amplitude in *T. h. helveticus* than in *T. v. vulgaris*. They also constitute a greater proportion of the display acts performed in the former species than in the latter.

* With the exception of the fan display, the similarity of form and transition of male acts suggests that qualitative differences in display and order of acts are not the primary source of information on which female choice of mate is based. Display is probably a means by which other species-specific sensory cues (e.g. differing odours) are transferred most effectively.

* Two models of sexual isolation are proposed: 1) a species recognition system (*sensu* Paterson 1985) in either males based on female morphology or odour, and/or in females based on male morphology or odour (but not display) and 2) where stimulation transferred during heterospecific male display fails to raise female receptivity at a high enough rate for spermatophore transfer to occur.

* Encounters between conspecific and hetreospecific *T. v. vulgaris* and *T. h. helveticus* females and *T. v. vulgaris* males appear to be initiated by chance in laboratory mating trials. No preferential sniffing behaviour at the start of these encounters was observed.

* Male *T. v. vulgaris* courted both conspecific and heterospecific females that were unrestrained and harnessed. Male *T. h. helveticus* showed extreme aversion to females of either species held in a harness.

* Females of both species perform more positive orientation acts in response to courting male *T. v. vulgaris* than they do to non-courting males; some degree of stimulation is clearly produced by heterospecific display. However, encounters between male *T. v. vulgaris* and heterospecific females did not progress beyond retreat display, which supports the hypothesis that heterospecific encounters fail as a consequence of inadequate stimulation of the female.

* The data suggest that female newts would benefit from a period of recovery of the effects of transfer into the aquarium prior to trials commencing.

* Male *T. v. vulgaris* remain in closer proximity and perform more positive acts in response to 'receptive' conspecific female behaviour over 'receptive' heterospecific female behaviour. No difference was found in response to the two groups of 'unreceptive' females. This suggests that preferential selectivity in males is dependant on a receptive motivational state in the female during courtship.

* A complex interactive process between potential partners appears to contribute to sexual isolation. This is based on the failure of the male to stimulate the female sufficiently and on a negative feedback system in which unresponsive behaviour in the female leads to a reduction in vigour of display in the male.

* Only male *T. h. helveticus* showed preferential selectivity for conspecific females over heterospecific females based on visual cues alone.

* Courtship behaviour was performed by males of both species towards females of both species in the visual cue trials. Although male *T. v. vulgaris* displayed with equal vigour to both groups, male *T. h. helveticus* paid more and longer visits to conspecific females and also performed more courtship acts towards them. These data suggest that visual cues may contribute to a specific mate recognition system in male *T. h. helveticus*, but not male *T. v. vulgaris*.

* None of the four groups showed aversion to heterospecific odour, but males of both species, and females which showed evidence of olfaction, all preferred the odour of conspecifics over heterospecifics. Failure to be attracted or stimulated by heterospecific chemical cues probably contributes to sexual isolation between the species.

* Newt odours clearly act as sexual attractants, however it is not known whether they also act as sexual stimulants; this would appear likely as male *T. vulgaris* displayed in the simultaneous presence of conspecific and heterospecific female odour alone.

* Where random choice is exhibited by female newts in tests requiring active selection of a potential stimulus (as in the visual and olfactory experiments), this should not be taken as conclusive evidence that these females are unable to discriminate, as the experiment is evaluated on a demand for an unnaturally strong female response.

* Females of both species preferred to remain in the vicinity of the *T. h. helveticus*-mode fan mimic. The preference of female *T. v. vulgaris* for the heterospecific fan display probably occurs because this faster fan speed produces a water current which is more stimulating that than produced by the slower, conspecific fan mimic.

* The fan mimic appears to produce a crude array of stimuli, some of which have been interpreted as potential prey.

* Sexual isolation is probably the consequence of a dual system whereby males fail to recognise the olfactory and/or visual cues produced by heterospecific females and so tend not to initiate courtship with them. Where interspecific courtship occurs however, the stimulation provided by the male is inadequate to raise female receptivity at a high enough rate. The evidence suggests that the olfactory and tactile cues produced and transferred by male display are probably important components of stimulation for females. It is possible that the colour and patterning of the male also contributes to female stimulation, but there is currently only circumstantial evidence to support this hypothesis.

* Sexual isolation between *T. v. vulgaris* and *T. h. helveticus* appears to be a by-product of two independant systems that have evolved to improve the efficiency of conspecific male and female newts meeting and mating.

Areas for future research

This study provides a brief investigation into the timing of courtship in *T. h. helveticus*; knowledge of the diel and seasonal mating activity of this species in the field is rudimentary and requires further investigation.

There are no documented reports of field investigations into the extent in which heterospecific encounters are initiated between *T. v. vulgaris* and *T. h. helveticus*. In particular the possible role of interspecific sexual interference is worthy of evaluation.

Sexual interference in *T. v. vulgaris* courtship encounters has been studied, unlike in *T. h. helveticus*. Verrell's (1984) laboratory experiments with the former species are worthy of extension to the latter;

these could then be followed by field studies to determine the extent in which it occurs under natural conditions.

That multiple mating and sperm storage occurs in female newts is well known. The importance of sperm competition in reproductive isolation has yet to be assessed in *Triturus*.

Knowledge of the ecology of terrestrial phase *T. v. vulgaris* and *T. h. helveticus* is far from complete, in particular there is very little known about the ecology of the eft. Investigations into habitat requirements and niche overlap of the two species would be particularly valuable for their conservation.

The viability of hybrids resulting from laboratory crosses between *T. helveticus* and the subspecies of *T. vulgaris* has been the subject of a number of studies. However, the value of these reports has been somewhat undermined because the conditions under which the larvae are reared are not equivalent to those in a field environment (e.g. there are no predators). Monitoring the success of hybrid larvae under natural or semi-natural conditions and comparing their viability to that of purebred larvae would give a more reliable indicator of relative fitness.

There has been much speculation about the function of the male dorsal crest, in particular its putative role as an auxilliary respiratory surface. Investigation of whether males with well developed secondary sexual characters are able to either remain submerged for longer, or are able to court females more vigorously, or for longer than males with poorly developed integumental features would produce valuable data.

Causal analysis of the homologous display acts in *T. v. vulgaris* and *T. h. helveticus* is almost complete with the exception of the Right Angle Tail Hold performed by *T. v. vulgaris* (Raxworthy 1989a). It would be interesting to carry out such an analysis (ref. Halliday 1975b) on this act to gain a better understanding of its function.

The study of sexual selection and mate choice in male *T. v. vulgaris* has been extensive, but not for male *T. h. helveticus* or females of these two species. In particular there is little evidence to support the popular hypothesis that sexual selection through female mate choice has led to divergence of male secondary sexual characteristics. Despite widespread speculation, it has not been determined whether females show a preference for males with better developed characteristics, possibly because of the difficulties in controlling for rate of male display. Comparing female response to normal and paedomorphic males (which do not possess the full range of male secondary sexual characteristics) is a worthwhile consideration.

As male *T. h. helveticus* show preferential selectivity for conspecific females based on visual cues and odour, I predict that males of this species would exhibit a preference to court conspecific females over heterospecific females when presented with both forms simultaneously under natural or semi-natural conditions.

The dorsal (or abdominal) gland in male newts shows extensive development during the breeding season and it has been hypothesised that it produces a chemical stimulant during *T. v. vulgaris* and *T. h. helveticus* courtship. Observing female response (conspecifics and heterospecifics) to normal males and males in which the dorsal gland had been removed or blocked would test this hypothesis.

In the tests on females in the olfactometer, there was evidence to suggest that females responded to conspecific male odours. Whether these odours act as a stimulant during courtship is not known, but could be investigated using nostril-plugged females exposed to courtship display.

The suggestion that fanning acts to transfer odours to the female is worthy of further attention. A tail mimic such as that used here, modified to allow odours to be piped down it would be one means by which this

suggestion could be tested. Modification of the tail could also be carried out to test for the importance of the tail filament in the hydrodynamics of display.

There is one other recorded subspecies of *T. helveticus*, *T. h. sequeri* from northern Spain and Portugal (Thorn 1968). Although it is reported to have muted characteristics compared to the nominal subspecies, no empirical comparison has been published relating to its morphology or courtship behaviour. Any variation in morphology may be reflected in quantitative or qualitative differences in display.

The visual and olfactory experiments carried out in this thesis could be extended to other closely related species of *Triturus*, e.g. *T. cristatus* and *T. marmoratus*. I predict that in species which do not hybridise, there would be relatively well developed olfactory or visual discrimination when compared to those that do hybridise.

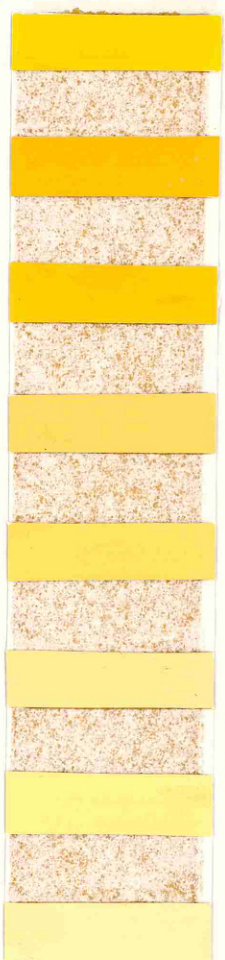
APPENDICES

APPENDIX I

Belly Colours - Approx. shades based on the Dulux Matchmaker Range 1982.

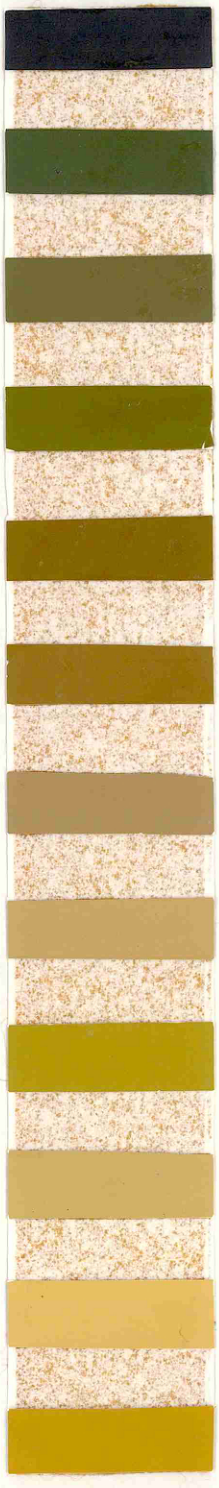
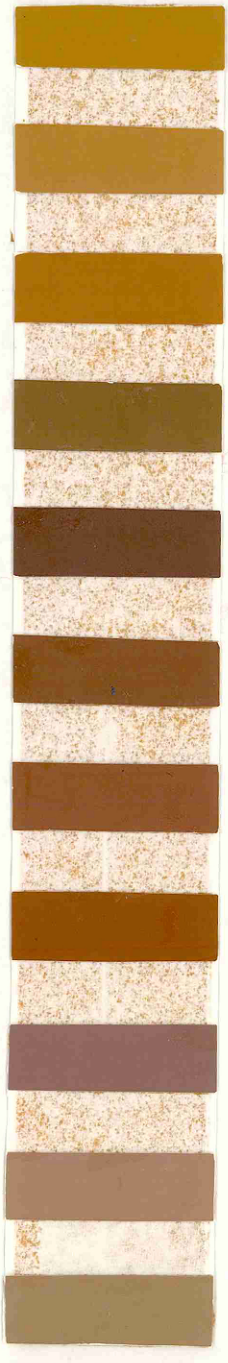
Colour Code	Shade
669	
737	
741	
646	
567	
587	
708	
897	
820	
734	

Colour Code	Shade
521	
753	
576	
837	
619	
778	
723	
869	



APPENDIX II

Body Colours - Approx. shades based on the Dulux Matchmaker Range 1982.

Colour Code	Shade	Colour Code	Shade
595		523	
724		871	
920		661	
678		574	
670		696	
552		892	
629		571	
744		551	
739		572	
505		784	
618		772	
775			

REFERENCES

Andrews, R.H. (1982). Mating behaviour and reproducive isolation of three species of reptile tick. *Anim. Behav.* 30:514-524.

Arnold, E.N., Burton, J.A. & Ovendon, D.W. (1978). A field guide to the reptiles and amphibians of Britain and Europe. London: Collins.

Arnold, S.J. (1972). The evolution of courtship behavior in salamanders. *Ph.D. Dissertation.* Ann Arbor: University of Michigan.

Arnold, S.J. (1977). The evolution of courtship behavior in New World salamanders with some comments on Old World salamanders. In: *The reproductive biology of amphibians.*, pp. 141-183, Taylor, D.H. & Guttman, S.I. (Eds.). New York: Plenum Press.

Arnold, S.J. & Houck, L.D. (1982). Courtship pheromones: evolution by natural and sexual selection. In: *Biochemical Aspects of Evolutionary Biology.*, pp. 173-211, Nitecki, M.H. (Ed.). Univ. of Chicago Press.

Arntzen, J.W. (1986a). Aspects of introgressive hybridisation in Crested and Marbled newts (Amphibia: Caudata). Preliminary results. In: *Studies in Herpetology.*, pp. 155-160, Rocek, Z. (Ed.). Prague: Charles University.

Arntzen, J.W. (1986b). Notes sur la coexistence d'espece sympatrique de tritons du genre *Triturus*. *Bull. Soc. Herp. France.* 37:1-8.

Auffenberg, W. (1965). Sex and Species Discrimination in Two Sympatric South American Tortoises. *Copeia* 3:335-342.

Ball, R.W. & Jameson, D.L. (1966). Premating isolating mechanisms in Sympatric and Allopatric *Hyla regilla* and *Hyla californiae*. *Evolution* 20:533-551.

Barton, N.H. & Hewitt, G.M. (1985). Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.* 16:113-148.

Bateman, A.J. (1948). Intra-sexual selection in *Drosophila*. *Heredity* 2:349-368.

Beebee, T.J.C. (1980). Belly colour and foot-webbing in newts of the genus *Triturus*. *Brit. J. Herp.* 6:59-61.

Beebee, T.J.C. (1981). Habitats of the British Amphibians (4). Agricultural Lowlands and a General Discussion of Requirements. *Biological Conservation* 21:127-139.

Bell, G. (1973). The life of the Smooth Newt *Triturus vulgaris* Linnaeus). *Ph.D. Thesis*. University of Oxford.

Belvedere, P., Colombo, L., Giacoma, C., Malacarne, G., & Andreoletti, G.E. (1988). Comparative and biochemical aspects of courtship pheromones in European newts. *Mon. Zool. Ital. (N.S.)* 22:397-403.

Bennazi, L. (1957). Sulla ibridazione fra *Triturus helveticus* e *Tr. vulgaris*. *Boll. Zool.* 24:235-242.

Berry, R.J. (1977). Inheritance and Natural History. *New Naturalist Series*. London: Collins.

Blab, J. & Blab, L. (1981). Quantitative Analysen zur Phanologie, Erfassbarkeit und Populationsdynamik von Molchbestanden des Kottenforstes bei Bonn (Amphibia: Caudata: Salamandridae). *Salamandra* 17:147-172.

Blair, W.F. (1955). Mating call and stage of speciation in the *Microhyla olivacea - M. carolinensis* complex. *Evolution* 9:469-480.

Blair, W.F. (1964). Evolution at population and interpopulational levels: isolating mechanisms and interspecies interactions in anuran amphibians. *Quart. Rev. Biol.* 39:334-344.

Blair, W.F. & Littlejohn, M.J. (1960). Stage of speciation of two allopatric populations of Chorus Frogs (*Pseudacris*). *Evolution* 14:82-87.

Brown, R.G.B. (1965). Courtship Behaviour in the *Drosophila obscura* group. Part II. Comparative Studies. *Behaviour* 25(3-4):281-323.

Brown, W.L. & Wilson, E.O. (1956). Character Displacement. *Syst. Zool.* 5:49-64.

Butlin, R. (1987). Speciation by reinforcement. *Trends Ecol. Evol.* 2(1):8-13.

Callan, H.G. & Spurway, H. (1951). A study of meiosis in intermediate hybrids of the newt *Triturus cristatus*. *Journal of Genetics* 50:235-249.

Carson, H.L. & Templeton, A.R. (1984). Genetic Revolutions in Relation to Speciation Phenomena: the Founding of New Populations. *Ann. Rev. Ecol.* Syst. 15:97-131.

Cedrini, L. & Fasolo, A. (1971). Olfactory attractants in sex recognition of the Crested newt. An electrophysiological research. *Monitore Zool. Ital. (N.S.)* 5:223-239.

Clifford, T. (1986). Notes on the morphometrics and spot patterns of female Smooth newts (*Triturus vulgaris*) at a coastal site in Lincolnshire. *Herpetological J.*, 1:93-96.

Collins, M.M. & Tuskes, P.M. (1979). Reproductive isolation in sympatric species of dayflying moths (*Hemileuca*: Saturnidae). *Evolution* 33(2):728-733.

Cooke, A.S. & Frazer, J.D. (1976). Characteristics of newt breeding sites. *J. Zool.*, London, 178:223-236.

Cooke, A.S. & Fulford, W.G. (1974). Observations on the feeding behaviour of a blind Warty Newt (*Triturus cristatus*). *British Journal of Herpetology* pp. 216.

Coyne, J.A., Orr, H.A. & Futuyma, D.J. (1988). Do we need a new species concept? *Syst. Zool.* 37(2):190-200.

Creed, K. (1964). A study of the newts in the New Forest. *British Journal Of Herpetology* 3:170-181.

Czopek, J. (1959). Skin and lung capillaries in European Common newts. *Copeia* 2:91-96.

Davis, W.C. & Twitty, V.C. (1964). Courtship behaviour and reproductive

isolation in the species of *Taricha* (Amphibia, Caudata). *Copeia* 4:601-610.

Darwin, C. (1871). The descent of man. London: Murray.

Dawley, E.M. (1984). Recognition of individual, sex and species odours by salamanders of the *Plethodon glutinosus - P. jordani* complex. *Anim. Behav.* 32:353-361.

Dawley, E.M. (1986). Behavioral isolating mechanisms in sympatric terrestrial salamanders. *Herpetologica* 42(2):156-164.

Dawley, E.M. (1987). Species discrimination between hybridizing and non-hybridizing terrestrial salamanders. *Copeia* 4:924-931.

Dawley, E.M. & Dawley, R.M. (1986). Species discrimination by chemical cues in a uni-sexual - bisexual complex of salamanders. *Journal of Herpetology* 20:116-119.

Dobzhansky, T. (1937). Genetic nature of species differences. *Am. Nat.* 71:404-420.

Dobzhansky, T. (1951). Genetics and the origin of species. (*3rd edition*, revised). New York and London: Columbia University Press.

Dobzhansky, T., Ayala, F.J., Stebbins, L. & Velentine, J.W. (1977). Evolution. San Francisco: Freeman.

Dolmen, D. (1981). Local migration, geotaxis and philopatry by *Triturus vulgaris* within a locality in central Norway. *Brit. J. Herp.* 6:151-158.

Dolmen, D. (1983). Diel rhythms of *Triturus vulgaris* and *T. cristatus* (Laurenti) (Amphibia) in Central Norway. *Det Kgl. Norske Videnskabers Selskab Museet.* Gunneria 42, Zoological Series No 13:1-34.

Ehrlich, P.R. (1961). Has the biological species concept outlived its usefulness? *Syst. Zool.* 10:167-176.

Emlen, S.T., Rising, J.D. & Thompson, W.I. (1975). A behavioral and morphological study of sympatry in the Indigo and Lazuli Buntings of the Great Plains. *The Wilson Bulletin* 87(2):145-179.

Finkler, W. (1923). Analytical studies on the factors causing the sexual display in the Mountain Newt (*Triton alpestris*). *Proc. Roy. Soc.* B. 95:356-364.

Ford, N.B. (1982). Species Specificity of Sex Pheromone Trails of Sympatric and Allopatric Garter Snakes (*Thamnophis*). *Copeia* 1:10-13.

Ford, N.B. (1984). Species specificity of sex pheromone trails in the Plains Garter snake, *Thamnophis radix*. *Herpetologica* 40(1):51-55.

Fouquette, M.J. (1975). Speciation in the Chorus Frogs I. Reproductive character displacement in the *Pseudacris nigrita* complex. *Syst. Zool.* 24(1):16-23.

Frazer, D. (1983). Reptiles and Amphibians in Britain. Glasgow: William Collins & Co. Ltd.

Freytag, G.E. (1950). Uber F1 bastarde zwischen Fadenmolch (*Triturus*

helveticus helveticus) und Teichmolch (*Triturus vulgaris*) insbesonddere uber Lantze bastarde mit *T. v. meridionalis*. *Abh. Ber. Naturk. Vorgesch.* 8:93-102.

Frost, J.S. & Bagnara, J.T. (1977). An analysis of reproductive isolation between *Rana magnaocularis* and *Rana berlandieri formeri* (*Rana pipiens* complex). *J. Exp. Zool.* 202(3):291-306.

Frost, J.S. & Platz, J.E. (1983). Comparative assessment of modes of reproductive isolation among four species of Leopard frogs (*Rana pipiens* Complex. *Evolution* 37 (1):66-78.

Futuyma, D.J. (1979). Evolutionary Biology. (*1st edition*). Sunderland, Massachusetts: Sinauer Associates Inc.

Foxon, G.E.H. (1964). Blood and Respiration. In: *Physiology of the amphibia.*, pp. 151-209, Moore, J.A. (Ed.). New York: Academic Press.

van Gelder, J.J. (1973). Ecological Observations On Amphibia In The Netherlands. II *Triturus helveticus* Razoumowski. Migration, hibernation and neoteny. *Netherlands Journal of Zoology* 23(1):86-108.

van Gelder, J.J. (1979). Differences in the sexual behaviour of the Smooth newt, *Triturus vulgaris* and the Palmate newt, *Triturus helveticus*. *Bull. Soc. Zool. France* 102, suppl. 2, Etho-ecologie des communications chez les amphibiens: 23-26.

Giacoma, C. & Sparreboom, M. (1987). On the sexual behaviour of the Italian newt, *Triturus italicus* (Caudata: Salamandridae). *Bijdr. Dierk.* 57:19-30.

Grant, P.R. (1975). The classical case of character displacement. Chapter 4 in: *Evolutionary Biology Vol. 8.*, Dobzhansky, T., Hecht, M.K. & Steere, W.C. (Eds.). New York: Plenum Press.

Grant, W.C. (1966). Endocrine induced courtship in three species of European newts. *Am. Zool.* 6:585.

Green, A.J. (1989). The Sexual Behaviour Of The Great Crested Newt, *Triturus cristatus* (Amphibia: Salamandridae). *Ethology* 83:129-153.

Greenfield, M.D. (1983). Reproductive isolation in Clearwing moths (Lepidoptera: Sesiidae): A tropical-temperate comparison. *Ecology* 64(2): 362-375.

Griffiths, R.A. (1984). Seasonal behaviour and intra-habitat movements in an urban population of Smooth newts *Triturus vulgaris* (Amphibia: Salamandridae). *J. Zool.*, London 203:241-251.

Griffiths, R.A. (1985). Diel profile of behaviour in the Smooth newt *Triturus vulgaris*: an analysis of environmental cues and endogenous timing. *Anim. Behav.* 33: 577-582.

Griffiths, R.A. (1986). Feeding niche overlap and food selection in Smooth and Palmate newts, *Triturus vulgaris* and *T. helveticus*, at a pond in mid-Wales. *J. Animal Ecol.* 55:201-214.

Griffiths, R.A. (1987). Microhabitat and seasonal niche dynamics of Smooth and Palmate newts, *Triturus vulgaris* and *T. helveticus*, at a pond in mid-Wales. *J. Animal Ecol.* 56:441-451.

Griffiths, R.A. & Mylotte, V.J. (1989). Observations on the development of secondary sexual characters of male newts *Triturus vulgaris* and *Triturus helveticus*. *J. Herp.* 22:476-480.

Griffiths, R.A., Roberts, J.M. & Sims, S. (1987). A natural hybrid newt *Triturus helveticus x T. vulgaris* from a pond in mid-Wales. *J. Zool.*, London 213:133-140.

Grusser-Cornehls, V. & Himstedt, W. (1978). The Urodele Visual System. In: *The amphibian visual system: A multi-disciplinary approach.*, pp. 203-266, Fite, K.V. (Ed.). New York: Academic Press.

Gwynne, D.T. & Morris, G.K. (1986). Heterospecific recognition and behavioral isolation in acoustic orthoptera (Insecta). *Evolutionary Theory* 8:33-38.

Hagstrom, T. (1977). Growth studies and ageing for adult *Triturus vulgaris* L. and *T. cristatus* Laurenti (Urodela, Salamandridae). *Zoologica scripta* 6:61-68.

Hagstrom, T. (1980). Egg production of newts (*Triturus vulgaris* and *T. cristatus*) in Southwestern Sweden. *ASRA Journal* 1(3):1-8.

Halliday, T. R. (1972). A study of the sexual behaviour of newts. *D. Phil. Thesis.* University of Oxford.

Halliday, T.R. (1974). Sexual behaviour of the Smooth newt *Triturus vulgaris* (Urodela, Salamandridae). *Journal of Herpetology* 8(4):277-292.

Halliday, T.R. (1975a). On the biological significance of certain morphological characters in males of the Smooth newt *Triturus vulgaris* and the Palmate newt *Triturus helveticus*. *Zool. J. Linn. Soc.* 56:291-300.

Halliday, T.R. (1975b). An observational and experimental study of sexual behaviour in the Smooth newt *Triturus vulgaris*. *Anim. Behav.* 23:291-322.

Halliday, T. R. (1976). The libidinous newt. An analysis of the variation in the sexual behaviour of the male Smooth newt, *Triturus vulgaris*. *Anim. Behav.* 24:398-414.

Halliday, T.R. (1977a). The courtship of European newts. An evolutionary perspective. In: *The Reproductive Biology of Amphibians.*, pp. 185-232, Taylor, D.H. & Guttman S.I. (Eds.). New York: Plenum Press.

Halliday, T.R. (1977b). The effect of experimental manipulation of breathing behaviour on the sexual behaviour of the Smooth newt *Triturus vulgaris*. *Anim. Behav.* 25:39-45.

Halliday, T.R. (1983). The study of mate choice. In: *Mate Choice.*, pp. 3-32, Bateson, P.P.G. (Ed.). Cambridge: Cambridge University Press.

Halliday, T.R. (In press). The evolution of courtship behaviour in newts and salamanders. *Advances in the Study of Behaviour.*

Halliday, T.R. & Joly, P. (1981). The functional significance of foot-webbing in European newts (*Triturus*). *Brit. J. Herp.* 6:131-132.

Halliday, T.R. & Sweatman, H.P.A. (1976). To breathe or not to breathe, the

newts problem. *Anim. Behav.* 24:551-561.

Halliday, T.R. & Verrell, P.A. (1988). Body Size and Age in Amphibians and Reptiles. *Journal of Herpetology* 22(3):253-265.

Halliday, T.R. & Worsnop, A. (1977). Correlation between activity and breathing rate in the Smooth newt *Triturus vulgaris* (Amphibia, Urodela, Salamandridae). *Journal of Herpetology.* 11(2) 244-246.

Halpern, M. (1980). Chemical ecology of terrestrial vertebrates. In: *Animals and Environmental Fitness.* Gilles R. (Ed). Oxford: Pergamon Press.

Harrison, J.D., Gittins, S.P. & Slater, F.M. (1983). The breeding migrations of Smooth and Palmate newts *Triturus vulgaris* and *T. helveticus* at a pond in mid-Wales. *J. Zool.*, London 199:249-258.

Harrison, J.D., Gittins, S.P. & Slater, F.M. (1984). Morphometric observations of Smooth and Palmate newts in mid-Wales. *Brit. J. Herp.* 6:410-413.

Highton, R. & Henry, S.A. (1970). Evolutionary interactions between species of North American salamanders of the genus *Plethodon.* In: *Evolutionary Biology., Vol. 4,* Dobzhansky, T., Hecht, M.K. & Steere, W.C. (Eds.). New York: Appleton-Century-Crofts.

Hillis, D.M. (1981). Premating isolating mechanisms among three species of the *Rana pipiens* complex in Texas and Southern Oklahoma. *Copeia* 2:312-319.

Himstedt, W. (1973). Die spektrale Empfindlichkeit von Urodelen in Abhangigkeit von Metamorphose, Jahreszeit und Lebenstraum. *Zool. Jahrb. Physiol.* 77:246-274.

Himstedt, W. (1979). The functional significance of color signals in partner recognition of the newt *Triturus alpestris*. *Copeia* 1:40-43.

Honma, K. & Tamaki, Y. (1976). Isolating Factors between the Smaller Tea Tortrix and the Summer Fruit Tortrix (Lepidoptera: Tortricidae) II. Sexual isolation. *Appl. Ent. Zool.* 11(3):202-208.

Houck, L.D., Arnold, S.J. & Hickman, A.R. (1988). Tests for sexual isolation in plethodontid salamanders (genus *Desmognathus*). *Journal of Herpetology* 22:186-191.

Houck, L.D. & Reagan, N.L. (1990). Male courtship pheromones increase female receptivity in a Plethodontid salamander. *Animal Behaviour 39:729-734.*

Hunsaker, D. (1962). Ethological Isolating Mechanisms in the *Sceloporus torquatus* group of lizards. *Evolution* 16:62-74.

Joly, P. (1981). Le comportement predateur du Triton Alpestre (*Triturus alpestris*). I. Etude descriptive. *Biology of Behaviour* 6:339-355.

Kawamura, T. (1950). Studies on hybridization in amphibians. III. Reciprocal hybrids between *Triturus pyrrhogaster* (BOIE) and *Triturus ensicauda* (HALLOWELL). *Jour. Sci. Hiroshima Univ.*, Ser B, Div. 1, 11:71-79.

Kawamura, T. & Sawada, S. (1959). On the sexual isolation among different

species and local races of Japanese newts. *J. Sci. Hiroshima Univ.* Ser. B. Div. 1, 18:17-30.

Kondrashev, S.L., Gnjubkin, V.F., Dimentman, A.M. & Orlov, O.J. (1976). Role of visual stimuli in the breeding behaviour of *Rana temporaria, Bufo bufo* and *Bufo viridis. Zool Zhurn.* 60:1027-1037.

Kroodsma, R.L. (1974). Species-recognition behavior of territorial male rose-breasted and black-headed Grosbeaks (*Pheuctus*). *The Auk* 91:54-64.

Lambert, D.M., Michaux, B. & White, C.S. (1987). Are species self defining? *Syst. Zool.* 36 (2):196-205.

Lande, R. (1980). Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution* 34:292-305.

Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA*, 78(6):3721-3725.

Lande, R. (1987). Genetic Correlation between the Sexes in the Evolution of Sexual Dimorphism and Mating Preferences. In:*Sexual Selection: Testing The Alternatives.*, Bradbury, J.W. & Andersson, M.B. (Eds), pp.83-94. John Wiley & Sons Ltd.

Lanier, G.N. & Burkholder, W.E. (1974). Pheromones in speciation of Coleoptera. In: *Pheromones.*, Birch, M.C. (Ed). North-Holland Research Monographs, Frontiers of Biology Vol. 32, pp.161-189. Amsterdam: North Holland Pub. Co.

Lantz, L.A. (1947). Hybrids between *Triturus cristatus* Laur. and *Triturus marmoratus* Latr. *Proc. Zool. Soc. Lond.* 117:247-258.

Lantz, L.A. & Callan, H.G. (1954). Phenotypes and spermatogenesis of interspecific hybrids between *Triturus cristatus* and *T. marmoratus*. *Journal of Genetics* 52:165-185.

Liley, N.R. (1966). Ethological Isolating Mechanisms in Four Sympatric Species of Poeciliid Fishes. *Behaviour*, Supplement XIII, pp. 1-197.

Liley, N. R. (1982). Chemical communication in fish. *Can. J. Fish Aquat. Sci.* 39:22-35.

Linnaeus, C. (1758). Systema naturae per regna tria naturae 10. Stockholm.

Littlejohn, M.J. (1965). Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). *Evolution* 19(2):234-243.

Littlejohn, M.J. (1969). The Systematic Significance of Isolating Mechanisms. *Systematic Biology*, pp. 459-482.

Littlejohn, M.J. & Loftus-Hills, J.J. (1968). An experimental evaluation of premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). *Evolution* 22:659-663.

Lofts, B. (1984). Amphibians. In: *Marshalls physiology of reproduction*. Lamming, G.E. (Ed.) 1:127-205. London: Churchill Livingstone.

Madison, D.M. (1977). Chemical communication in amphibians and reptiles.

In: *Chemical Signals In Vertebrates.*, pp. 135-168. Muller-Schwarze, D. & Mozell, M.M. (Eds.). New York: Plenum Press.

Malacarne, G. & Cortassa, R. (1983). Sexual selection in the Crested newt. *Anim. Behav.* 31:1256-1570.

Malacarne, G. & Giacoma, C. (1980). Effect of lesions to the rostral preoptic area on courtship behaviour in the male Crested newt, *Triturus cristatus carnifex* (Laur). *Monitore Zool. Ital. (N.S.)* 14:9-17.

Malacarne, G. & Vellano, C. (1982). Effects of nostril plugging and habenulectomy on sexual behaviour in the male Crested newt. *Behavioural Processes* 7:307-317.

Malacarne, G. & Vellano, C. (1987). Behavioural evidence of a courtship pheromone in the Crested newt, *Triturus cristatus carnifex.* *Copeia* 1987:245-247.

Malacarne, G., Bottoni, L., Massa, R. & Vellano, C. (1984). The abdominal gland of the Crested newt: a possible source of courtship pheromones. Preliminary ethological and biochemical data. *Monitore Zool. Ital. (N.S.)* 18:33-39.

Margolis, S.E. (1982). Buccal movements as a component of orienting behaviour in the newts *Triturus vulgaris* and *T. cristatus.* Zoologicheskii *Zhurnal Moscow* 56(4):564-575.

Markow, T.A. (1981). Courtship behaviour and control of reproductive isolation between *Drosophila mojavensis* and *Drosophila arizonensis.*

Evolution 35(5):1022-1026.

Marquenie, J.G.M. (1950) De balts van de kleine watersalamander. *De Lev. Nat.* 53:147-155.

Mayr, E. (1940). Speciation phenomena in birds. *Am. Nat.* 74:249-278.

Mayr, E. (1963). Animal species and evolution. Cambridge, Massachusetts: Harvard University Press.

Mayr, E. (1976). Evolution and the diversity of life. Selected essays. Cambridge, Massachusetts: The Belknap Press of Harvard University Press.

Mecham, J.S. (1961). Isolating mechanisms in anuran amphibia. In: *Vertebrate speciation.*, Blair, W.F. (Ed.). Austin: University of Texas Press.

Michaud, T.C. (1962). Call discrimination by females of the chorus frogs, *Pseudacris clarki* and *Pseudacris nigrita. Copeia* 1962:213-215.

Moller, A. (1951). Die struktur des augefbei urodelen vershiedener korpergrose. *Zool. Jahrb. Abt. Allq. Zool. Physiol. Tiere.* 62:138-182.

Muller-Schwarze, D. (1974). Olfactory recognition of species, groups, individuals and physiological states among mammals. In: *Pheromones.*, Birch, M.C. (Ed). North-Holland Research Monographs, Frontiers of Biology Vol. 32, pp.316-326. Amsterdam: North Holland Pub. Co.

Muller, H.J. (1939). Reversibility in evolution considered from the standpoint of genetics. *Biological Reviews* 14:261-280.

Muller, H.J. (1942). Isolating mechanisms, evolution and temperature. *Biol. Symp.* 6:71-125.

Nelson, J.B. (1959). The courtship of *Triturus cristatus*. *Honours thesis*. St. Andrews University.

Nelson, J.B. (1960). Courtship display in the Italian Crested Newt (*Triturus cristatus carnifex*). *Biol. J. (St. Andrews University)* 1:6-9.

Nie, N.H., Hull, C.H. Jenkins, J.G., Steinbrenner, K. & Bent, D.H. (1975). Statistical Package for the Social Sciences (2nd edition). New York: McGraw-Hill.

Nei, M., Maruyama, T. & Wu, C. (1983). Models of evolution of reproductive isolation. *Genetics* 103:557-579.

Noble, G.K. (1931). The biology of the amphibia. New York: McGraw-Hill.

Passmore, N.I. (1981). The relevance of the specific mate recognition concept to anuran reproductive biology. *Monitore Zool. Ital.* 6:93-108.

Passmore, N.I. (1981). Sound levels of mating calls of some african frogs. *Herpetologica* 37(3):166-171.

Paterson, H.E.H. (1978). More evidence against speciation by reinforcement. *African Journal of Science* 74 369-371.

Paterson, H.E.H. (1985). The recognition concept of species. In: *Species and Speciation*., Vrba, E.S. (Ed.). Transvaal Museum Monograph No4: 21-29.

Pretoria: Transvaal Museum.

Patterson, J.T. & Stone, W.S. (1952). Evolution in the genus *Drosophila*. New York: Macmilan.

Pecio, A. & Rafinski, J. (1985). Sexual behaviour of the Montandon's newt *Triturus montandoni* (Boulenger) (Caudata: Salamandridae). *Amphibia-Reptilia* 6:11-22.

Perrigo, G. & Bronson, F.H. (1982). Signaling and Priming Communication: Independent Roles in the Reproductive Isolation of Spatially-Separated Populations of Rodents. *Behavioral Ecology and Sociobiology* 10:181-184.

Persson, O. (1946). Studier over konskvoten hos vattenodlor. *K. Fysiogr. Sallsk. Lund. Forh.* 16:218-224.

Prechtl, H.F.R. (1951). Zur paarungsbiologie einiger mocharten. *Zeit. Tierpsychol.* 8(3):337-348.

Rafinski, J. & Arntzen, J.W. (1987). Biochemical aspects of the Old World newts, genus *Triturus*. Isozyme data. *Herpetologica* 43(4):446-457.

Ratcliffe, L.M. & Grant, P.R. (1983). Species recognition in Darwin's finches (*Geospiza*, Gould). I. Discrimination by morphological cues. *Animal Behaviour* 31(4):1139-1153.

Raxworthy, C.J. (1989a). The subspecific evolution of courtship behaviour and sexual dimorphism in the Smooth newt *Triturus vulgaris*. *Ph. D. Thesis*, Open University.

Raxworthy, C.J. (1989b). Courtship, Fighting and Sexual Dimorphism Of The Banded Newt, *Triturus vittatus ophryticus*. *Ethology* 81:148-170.

Razoumowsky, G. (1789). Histoire naturelle du Jorat et de ses environs. Lausanne.

Roberts, J.M. & Verrell, P.A. (1984). The physical abnormality of the limbs of Smooth newts (*Triturus vulgaris*). *Brit. J. Herp.* 6(11):416-418.

Roelofs, W.L. & Carde´, R.T. (1974). Sex pheromones in the reproductive isolation of lepidopterous species. In: *Pheromones.*, Birch, M.C. (Ed). North-Holland Research Monographs, Frontiers of Biology Vol. 32, pp.96-114. Amsterdam: North Holland Pub. Co.

Salmon, M. (1965). Waving Display and Sound Production in the Courtship Behavior of *Uca pugilator* with Comparisons to *U. minax* and *U. pugnax*. *Zoologica* 50(3):123-150.

Salmon, M., Ferris, S.D., Johnston, D., Hyatt, G. & White, G.S. (1979). Behavioral and Biochemical Evidence For Species Distinctiveness In The Fidler Crabs *Uca speciosa* and *U. spinicarpa*. *Evolution* 33(1):182-191.

Salthe, S.N. & Mecham, J.S. (1974). Reproduction and courtship patterns. In: *Physiology of the amphibia.*, 2:309-521, Lofts, B. (Ed.). New York: Academic Press.

Sanders, C.J. (1971). Daily activity patterns and sex pheromone specificity as sexual isolating mechanisms in two species of *Choristoneura* (Lepidoptera: Tortricidae). *The Canadian Entomologist*, April 1971,

103:498-502.

Sawada, S. (1963a). Studies on the local races of the Japanese newt, *Triturus pyrrhogaster* Boie. I. Morphological characters. *J. Sci. Hiroshima Univ. Ser. B*, Div. 1, 21:135-166.

Sawada, S. (1963b). Studies on the local races of the Japanese newt, *Triturus pyrrhogaster* Boie. II. Sexual isolation mechanisms. *J. Sci. Hiroshima Univ. Ser. B*, Div. 1, 21:167-180.

Scali, V. & Mancino, G. (1968). Observazioni sulla spermatogenesi di ibridi poliploidi *Triturus helveticus / vulgaris* female *x T. vulgaris* male. *Atti. Soc. Tosc. Sc. Nat.* 75:8-18.

Schaller, F. & Schwalb, H. (1961). Attrappenversuche mit Larven und Imagines einheimischer Leuchtkafer (Lampyrinae). *Zool. Anz.*, Suppl. 24:154-166.

von Schilcher, V. & Dow, M. (1977). Courtship behaviour in *Drosophila*: Sexual Isolation or Sexual Selection. *Z. Tierpsychol.* 43:304-310.

Schmid, M., Olert, J. & Klett, C. (1979). Chromosome Banding in Amphibia. III. Sex chromosomes in *Triturus*. *Chromasoma* (Berl) 71:29-55.

Schmidtler, J.J. & Schmidtler, J.F. (1967). Uber die Verbreitung der Molchgattung *Triturus* in Kleinasien. *Salamandra* 3:15-36.

Siegel, S. & Castellan, N.J. (1988). Nonparametric Statistics for the Behavioural Sciences. (*2nd edition*), New York, London: McGraw-Hill.

Schoorl, J. & Zuiderwijk, A. (1981). Ecological isolation in *Triturus cristatus* and *Triturus marmoratus* (Amphibia: Salamandridae). *Amphibia-Reptilia* 3/4:235-252.

Smith, N.G. (1966). Evolution of some Arctic Gulls (*Larus*): An experimental study of isolating mechanisms. *Ornithological Monographs* 4:1-99. American Ornithologists' Union.

Smith, M. (1973). The British Amphibians and Reptiles. *New Naturalist Series 20*, 5th edition. London: Collins.

Snyder, W.F. & Jameson, D.L. (1965). Multivariate geographic variation of mating call in populations of Pacific tree frog (*Hyla regilla*). *Copeia* 1965:129-142.

Sokal, R.R. & Crovello, T.J. (1970). The biological species concept: A critical evaluation. *Am. Nat.* 104:127-153.

Sparreboom, M. (1986). Courtship in newts of the genus *Triturus* and *Paramesotriton*. In: *Studies in Herpetology*., Rocek, Z. (Ed.), 529-534. Prague: Charles University.

Sparreboom, M. & Arntzen, J. W. (1987). A survey of behavior in the Old World newts (genus *Triturus*). *Proc. 4th. Ord. Gen. Meet. S.E.H.*, Nijmegen 1987:369-372.

Spurway, H. & Callan, H.G. (1960). The vigor and male sterility of hybrids between the species *Triturus vulgaris* and *T. helveticus*. *Journal of Genetics* 57(1):84-118.

Steward, J.W. (1969). The tailed amphibians of Europe. Newton Abbot: David and Charles.

Teyssedre, C. & Halliday, T.R. (1986). Cumulative effect of the male's displays in the sexual behaviour of the Smooth newt, *Triturus vulgaris* (Urodela, Salamandridae). *Ethology* 71:89-102.

Thorn, R. (1968). Les salamandres d'Europe, d'Asie et d'Afrique du Nord. Paris: Lechavalier.

Tinbergen, N. (1953). Social behaviour in animals. London: Methuen & Co. Ltd.

Tinbergen, N. & Ter Pelkwijl, J.J. (1938). De kleine watersamander. *De Lev. Nat.* 43:232-237.

Tinsley, R.C., Kobel, H.R. and Fischberg, M. (1979). The biology and systematics of a new species of *Xenopus* (Anura: Pipidae) from the highlands of Central Africa. *J. Zool.*, London 188:69-102.

Trivers, R.L. (1972). Parental investment and sexual selection. In: *Sexual selection and the Decent of Man, 1871-1971.*, pp. 136-179, Campbell, B. (Ed.). Chicago: Aldine Pub. Co.

Twitty, V.C. (1936). Correlated genetic and embryological experiments on *Triturus*. I. Hybridization: Development of three species of *Triturus* and their hybrid combinations. II. Transplantation: The embryological basis of species differences in pigment pattern. *J. Exp. Zool.*, 74:239-302.

Twitty, V.C. (1955). Field experiments on the biology and genetic relationships of the Californian species of *Triturus*. *J. Exp. Zool.* 129:129-147.

Twitty, V.C. (1961a). Experiments on homing behavior and speciation in *Taricha*. In: *Vertebrate Speciation: a symposium*., pp. 415-459. Austin, Texas: Univ. of Texas Press.

Twitty, V.C. (1961b). Second-generation hybrids of the species of *Taricha*. *Proc. Nat. Acad. Sci.* 47:1461-1486.

Twitty, V.C. (1964). Fertility of *Taricha* species hybrids and viability of their offspring. *Proc. Nat. Acad. Sci.* 51:156-161.

Twitty, V.C. & Bodenstein, D. (1939). Correlated genetic and embryological experiments on *Triturus*. III. Further transplantation experiments on pigment development. IV. The study of pigment cell behavior *in vitro*. *J. Exp. Zool.* 81:357-398.

Ultsch, G.R. (1976). Respiritory surface area as a factor controlling the standard rate of oxygen consumption of aquatic salamanders. *Resp. Physiol.* 26:357-369.

Veith, M. & Dorr, L. (1985). Zur Variabilitat morphognoshscher Artmerkmale in mitteleuropaischen Teich-und Fadenmolch - Populationen, *Triturus vulgaris vulgaris* (Linnaeus, 1758) und *Triturus helveticus helveticus* (Razoumowski, 1789). *Salamandra* 21(2/3):197-218.

Verrell, P.A. (1982). Newts can be colorful too. *Bioscience* 32:244.

Verrell, P.A. (1983). Sexual behaviour and sexual selection in newts. *Ph.D. Thesis*, Open University.

Verrell, P.A. (1984). Sexual interference and sexual defense in the Smooth newt, *Triturus vulgaris* (Amphibia, Urodela, Salamandridae). *Z. Tierpsychol.* 66:242-254.

Verrell, P.A. (1985). Male mate choice for large, fecund females in the Red-spotted newt *Notophthalmus viridescens*; how is size assessed? *Herpetologica* 41:386-395.

Verrell, P.A. (1986a). Limited male mating capacity in the Smooth newt, *Triturus vulgaris vulgaris* (Amphibia). *J. comp. Physcol.* 100:291-295.

Verrell, P.A. (1986b). Male discrimination for Larger, More Fecund Females in the Smooth Newt, *Triturus vulgaris*. *Journal of Herpetology* 20(3):416-422.

Verrell, P.A. (1988). Stabilizing selection, sexual selection and speciation: a view of specific-mate recognition systems. *Syst. Zool.* 37:209-215.

Verrell, P.A. (1989). An experimental study of the behavioral basis of sexual isolation between two sympatric Plethodontid salamanders, *Desmognathus imitator* and *D. ochropaeus*. *Ethology* 80:274-282.

Verrell, P.A. & Arnold, S.J. (1989). Behavioral observations of sexual isolation among allopatric populations of the Mountain Dusky Salamander *Desmognathus ochrophaeus*. *Evolution* 43:(4),745-755.

Verrell, P.A. & Halliday, T.R. (1985). Reproductive dynamics of a population of Smooth newts, *Triturus vulgaris*, in southern England. *Herpetologica* 41(4):386-395.

Verrell, P.A., Halliday, T.R. & Griffiths, M. (1986). The annual reproductive cycle of the Smooth newt (*Triturus vulgaris*) in England. *J. Zool.*, London 210:101-119.

Verrell, P.A. & McCabe, N. (1988). Field observations on the sexual behaviour of the Smooth newt, *Triturus vulgaris vulgaris* (Amphibia: Urodela: Salamandridae). *J. Zool.*, London 214:533-545.

Verrell, P.A. & Francillion, H. (1986). Body size, age and reproduction in the Smooth Newt, *Triturus vulgaris*. *J. Zool.*, Lond. (A) 210:89-100.

Waage, J.K. (1975). Reproductive isolation and the potential for character displacement in the damselflies *Calopteryx maculata* and *C. aequabilis* (Odonata: Calopterygidae). *Systematic Zoology* 24(1):24-36.

Wallis, G.P. & Arntzen, J.W. (1989). Mitochondrial DNA variation in the Crested newt superspecies: limited cytoplasmic geneflow among species. *Evolution* 43:88-104.

Wambreuse, P. & Bels, V. (1984). Analyse qualitative et quantitative de la parade sexuelle du triton palme *Triturus helveticus* (Razoumowsky 1798). *Cahiers d'Ethologie appliquee* 4(4):193-218.

Watson, G.F. (1972). The *Litoria ewingi* Complex (Anura: Hylidae) in South-Eastern Australia. II. Genetic incompatability and delimination of a narrow

hybrid zone between *L. ewingi* and *L. paraewingi*. *Australian Journal Zoology* 20:423-433.

Williams, E.E. & Rand, A.S. (1977). Species recognition, dewlap function and faunal size. *Am. Zool.* 17:261-270.

White, M.J.D. (1978). Modes of speciation. San Franscisco: W.H. Freeman & Co.

Wilz, K.T. (1970). Reproductive isolation in Two Species of Stickleback (Gasterosteidae). *Copeia* 3:587-590.

Wolterstorff, W. & Freytag, G.E. (1951). Eine studie uber das verwandtschaftsverhatnis von Teichmolch (*Triturus vulgaris*) und Fadenmolch (*Triturus helveticus*). *Abh. Ber. Naturkd. Vorgesch.* , Magdeburg 8 (4) 139-196.

Zippelius, H.M. (1948). Untersuchungen uber das Balzverhalten heimisher Molche. *Verh. Dtsch. Zool. Ges. Kiel.* pp.127-130.

Zuiderwijk, A. & Sparreboom, M. (1986). Territorial behaviour in Crested Newt *Triturus cristatus* and Marbled Newt *Triturus marmoratus* (Amphibia - Urodela). *Bijdragen tot de Dierkunde* 56:205-213.